JEM

Supplemental material

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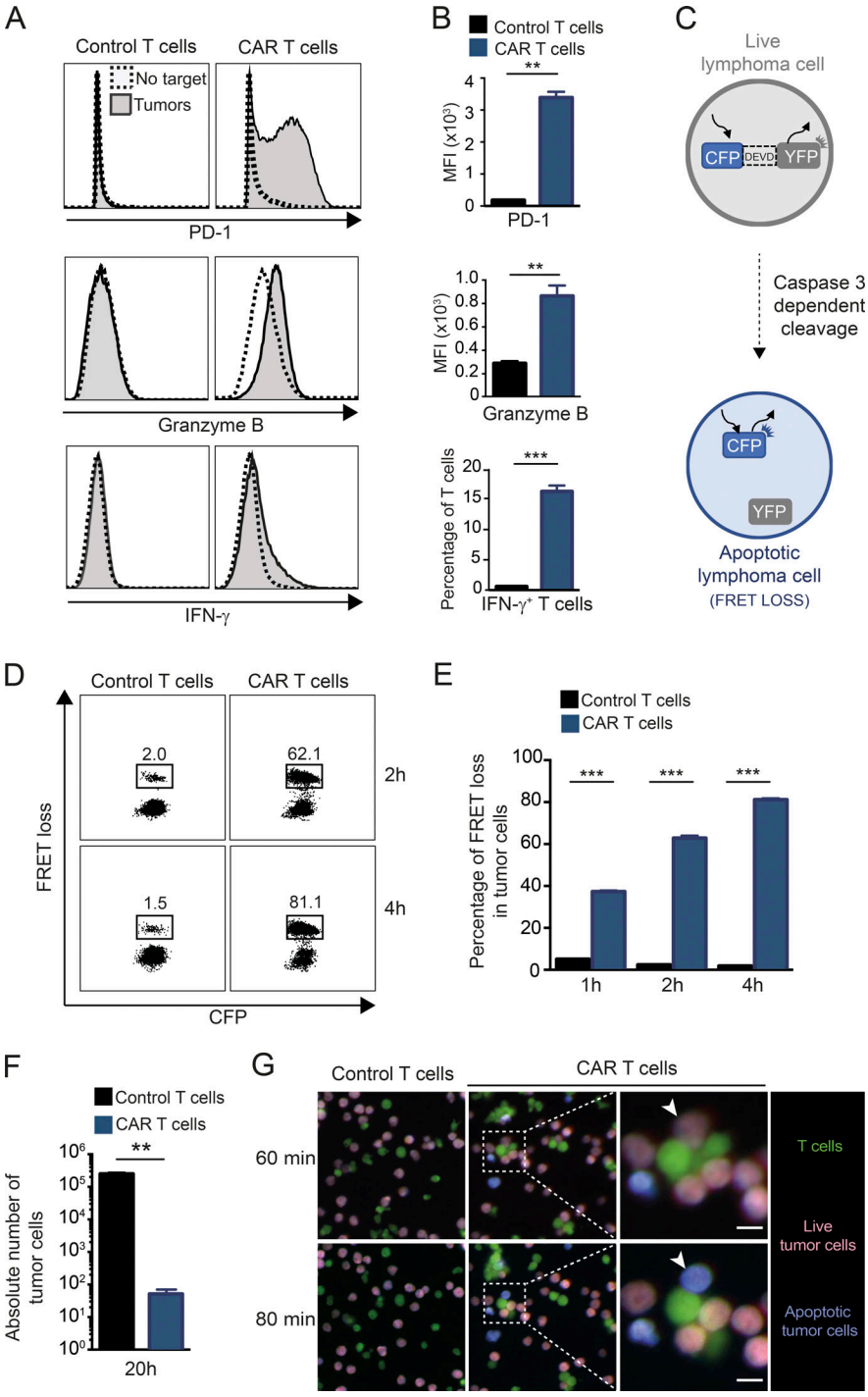


Figure S1. **Detecting CAR T cell–mediated tumor killing using a genetically encoded apoptosis reporter. (A and B)** Purified hCD34⁺ CAR or untransduced CD8⁺ T cells (activated in similar conditions) were cultured 20 h in the presence or absence Eμ-myc-DEVD malignant B cells at a 1:1 ratio. PD-1, intracellular Granzyme B, and IFN-γ expression was assessed by flow cytometry for CAR and control T cells. Data are shown as mean ± SEM. MFI, mean fluorescence intensity. Unpaired *t* test was used for statistical analysis. **, P < 0.01; ***, P < 0.001. **(C)** Schematic of the FRET-based caspase-3 reporter used in Eμ-myc-DEVD malignant B cells. CFP and YFP are linked by the caspase target peptide bearing the DEVD sequence. Caspase-3 activity during apoptosis results in FRET loss. **(D)** Representative FACS plots showing FRET loss in Eμ-myc-DEVD cells upon co-culture with CAR T cells (1:1 ratio), but not in the presence of control CD8⁺ T cells. **(E)** Bar chart showing the quantification of FRET loss in tumor cells at the indicated time points. Data are shown as mean ± SEM. Unpaired *t* test with Holm–Sidak correction for multiple comparisons was used for statistical analysis. ***, P < 0.001. **(F)** Absolute tumor cell numbers following 20 h co-culture of 5 × 10^5 Eμ-myc-DEVD malignant B cells with 5 × 10^5 CAR or control T cells. Data are shown as mean ± SEM. Unpaired *t* test was used for statistical analysis. **, P < 0.01. Representative of two independent experiments. **(G)** Visualizing CAR T cell–induced tumor apoptosis in vitro. T cells were incubated with Eμ-myc-DEVD cells. Representative images showing CAR or control T cells in green, live tumors cells in pink, and apoptotic tumor cells in blue. Insets highlight that CAR T cells, but not control T cells, induce tumor cell apoptosis during cell–cell interactions (arrowheads). Scale bars represent 10 μm.

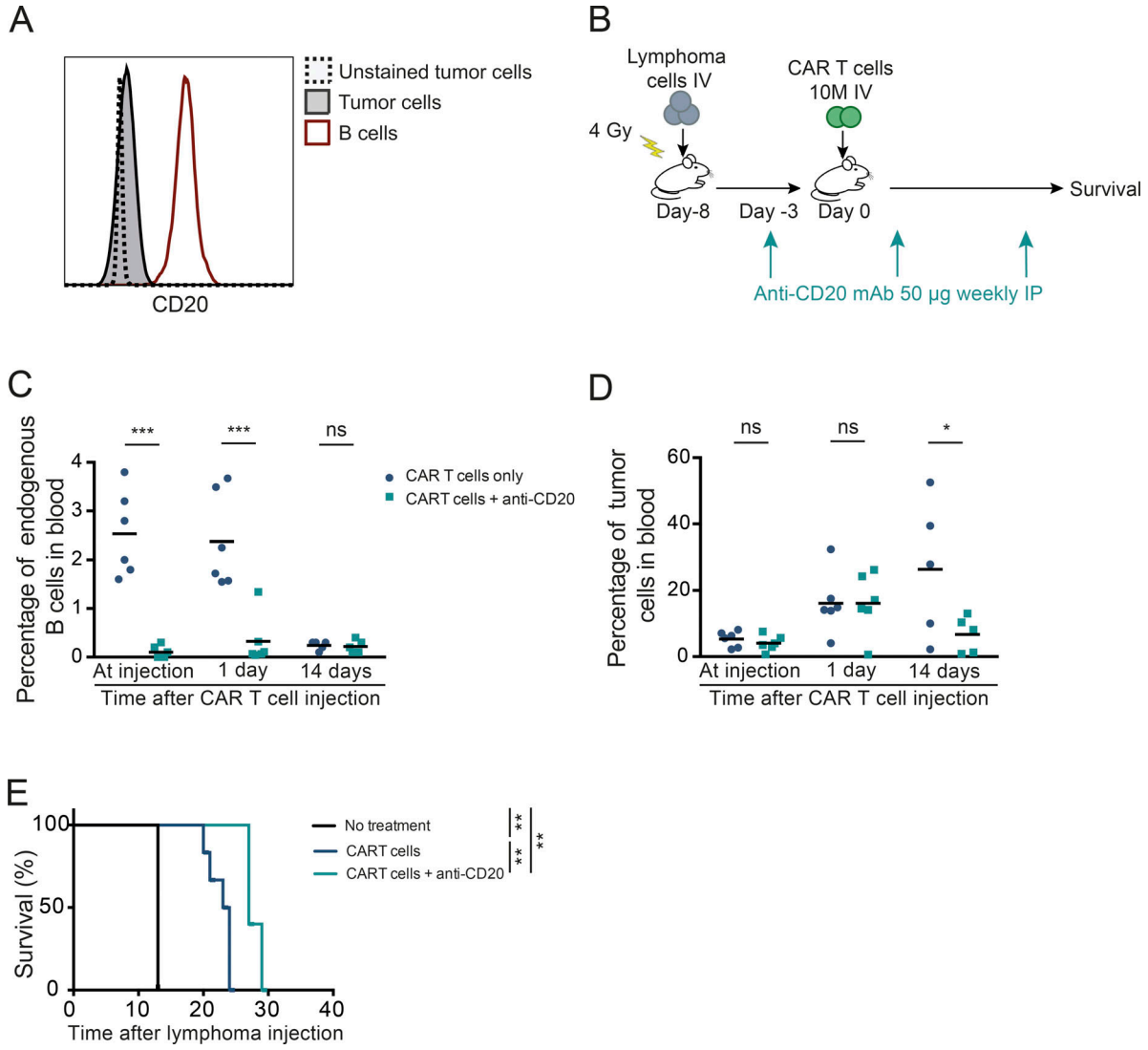


Figure S2. **Removal of circulating B cells increases CAR T cell activity. (A)** Flow cytometry histograms showing that B cells, but not Eμ-myc tumor cells, express detectable levels of CD20. **(B–E)** B cell lymphomas were established by i.v. injection of 0.5 × 10^6 Eμ-myc-DEVD cells in C57BL/6 mice after sublethal irradiation (4 Gy). 8 d later, mice were injected i.v. with 10 × 10^6 CAR T cells. Anti-CD20 antibody was injected 3 d before CAR T cell transfer and subsequently every week. **(B)** Experimental setup. **(C)** Efficient depletion of circulating B cells upon anti-CD20 treatment. **(D)** Anti-CD20 treatment did not deplete circulating tumors but enhanced CAR T cell therapy at 2 wk. Blood tumor burden as determined by flow cytometry is graphed for the indicated time points for CAR T cell and CAR T cell + anti-CD20–treated recipients. For data presented in C and D, each dot represents one mouse. Unpaired *t* test with Holm–Sidak correction for multiple comparisons was used for statistical analysis. ***, P < 0.001; *, P < 0.05; ns, not significant. **(E)** Combined treatment using CAR T cell and anti-CD20 antibody significantly extends recipient survival. Log-rank test was used for statistical analysis. **, P < 0.01.

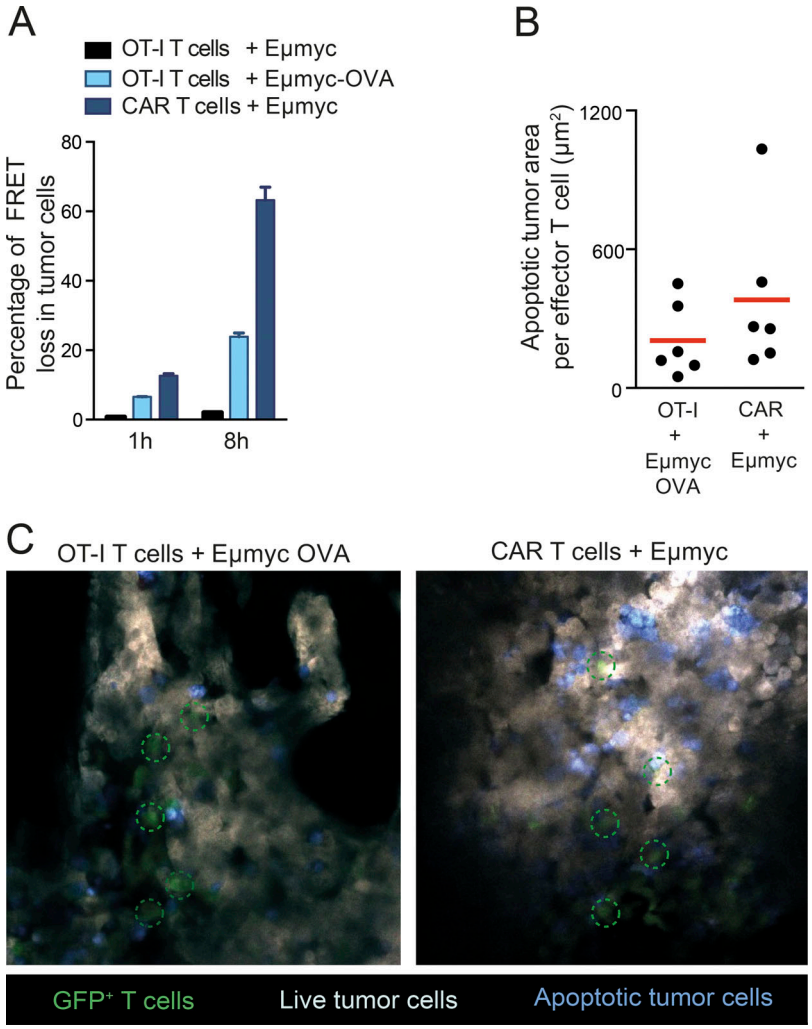
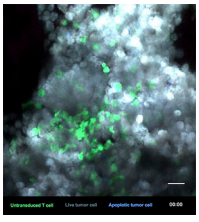
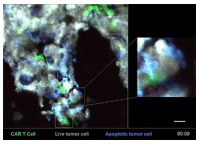


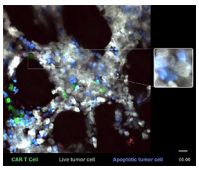
Figure S3. **Comparing CAR T cell and OT-I T cell killing in vitro and in vivo. (A)** In vitro killing potential of CAR T and OT-I T cells. Activated OT-I T cells were cultured with Eμ-myc-DEVD or Eμ-myc-OVA-DEVD cells. CAR T cells were cultured with Eμ-myc-DEVD cells. Apoptosis in tumor cells as measured by FRET loss was determined at the indicated time points using flow cytometry. Data are shown as mean ± SEM. **(B and C)** In vivo killing potential of CAR T and OT-I T cells. Eμ-myc-DEVD or Eμ-myc-OVA-DEVD B cell lymphomas were established in $Rag2^{-/-}$ mice conditioned by sublethal irradiation. On day 10, CAR T cells were injected in Eμ-myc-DEVD bearing recipients and activated OT-I in Eμ-myc-OVA-DEVD. Intravital imaging was performed on day 2 after effector T cell transfer. **(B)** Quantification of the area of apoptotic tumors normalized per T cell present in the image. Each dot represents one imaged tumor area. Data are compiled from two individual mice. **(C)** Representative two-photon images showing the presence of apoptotic tumors 2 d after CAR T cells or OT-I T cells transfer. Effector T cells are shown in green, live tumor cells in gray, and apoptotic tumor cells in blue. Green circles highlight effector T cells. Data are representative of two independent experiments.



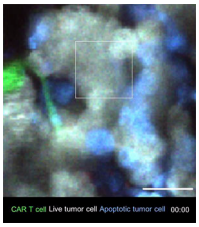
Video 1. **Intravital bone marrow imaging of lymphoma-bearing mice treated with control T cells.** C57BL/6 mice were sublethally irradiated and injected with Eμ-myc-DEVD tumor cells 7 d before treating them with untransduced GFP^+ activated $CD8^+$ T cells (control cells). Mice were subjected to intravital bone marrow imaging 40 h after T cell transfer. Live tumor cells appear in gray, apoptotic tumor cells in blue, and T cells in green. Scale bar represents 20 μm. Total duration = 27 min.



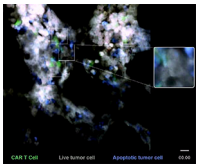
Video 2. **Efficient and rapid killing of B cell tumors by CAR T cells in the bone marrow (example 1).** C57BL/6 mice were sublethally irradiated and injected with Eμ-myc-DEVD tumor cells 7 d before treating them with GFP$^+$ CAR T cells. Mice were subjected to intravital bone marrow imaging 40 h after CAR T cell transfer. Live tumor cells appear in gray, apoptotic tumor cells in blue, and T cells in green. Squares highlight tumor cell apoptosis during direct contacts with CAR T cells. Scale bar represents 20 μm. Total duration = 64 min.



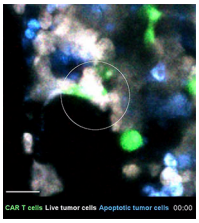
Video 3. **Efficient and rapid killing of B cell tumors by CAR T cells in the bone marrow (example 2).** C57BL/6 mice were sublethally irradiated and injected with Eμ-myc-DEVD tumor cells 7 d before treating them with GFP$^+$ CAR T cells. Mice were subjected to intravital bone marrow imaging 40 h after CAR T cell transfer. Live tumor cells appear in gray, apoptotic tumor cells in blue, and T cells in green. Squares highlight tumor cell apoptosis during direct contacts with CAR T cells. Scale bar represents 20 μm. Total duration = 60 min.



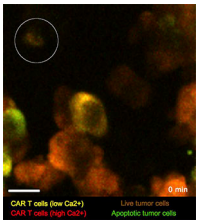
Video 4. **Example of a B cell tumor undergoing apoptosis without apparent CAR T cell contacts.** C57BL/6 mice were sublethally irradiated and injected with Eμ-myc-DEVD tumor cells 7 d before treating them with GFP$^+$ CAR T cells. Mice were subjected to intravital bone marrow imaging 40 h after CAR T cell transfer. The square highlights a tumor cell undergoing apoptosis in the absence of apparent interactions with CAR T cells. Scale bar represents 20 μm. Total duration = 64 min.



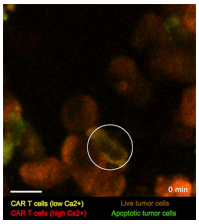
Video 5. **CAR T cell serial killing of adjacent tumor cells in the bone marrow.** C57BL/6 mice were sublethally irradiated and injected with Eμ-myc-DEVD tumor cells 7 d before treating them with GFP$^+$ CAR T cells. Mice were subjected to intravital bone marrow imaging 40 h after CAR T cell transfer. Live tumor cells appear in gray, apoptotic tumor cells in blue, and T cells in green. The inset highlights a CAR T cell killing two adjacent tumor cells. Additional squares show examples of CAR T cell–mediated tumor apoptotic events. Scale bar represents 20 μm. Total duration = 64 min.



Video 6. **Example of a CAR T cell forming prolonged interactions with tumor cells without inducing their apoptosis.** C57BL/6 mice were sublethally irradiated and injected with Eμ-myc-DEVD tumor cells 7 d before treating them with GFP$^+$ CAR T cells. Mice were subjected to intravital bone marrow imaging 40 h after CAR T cell transfer. Live tumor cells appear in gray, apoptotic tumor cells in blue, and T cells in green. Scale bar represents 20 μm. Total duration = 78 min.



Video 7. **Example of CAR T cell with high calcium responses upon interactions with tumor cells.** B cell lymphomas were established by i.v. injection of 0.5 × 10^6 Eμ-myc-DEVD cells in C57BL/6 mice after sublethal irradiation (4 Gy). 7 d later, mice were injected i.v. with 20 × 10^6 purified CAR T cells expressing the Twitch2B genetically encoded calcium indicator. Bone marrow intravital imaging was performed 40 h after T cell transfer. The highlighted CAR T cell (white circle) exhibited a first calcium increase after a contact with a tumor cell (without killing) and a second calcium responses (associated with killing; see arrow) upon encountering a new target. Low and high calcium concentrations are shown in yellow and red, respectively. Tumor cells are shown in brown when alive and green when undergoing apoptosis. Scale bar represents 10 μm. Total duration = 37 min.



Video 8. **Example of CAR T cell with weak calcium responses upon interactions with tumor cells.** The highlighted CAR T cell (white circle) established tight contacts with various tumor cells that did not induce sustained calcium signals nor target cell killing. Scale bar represents 10 µm. Total duration = 28 min.