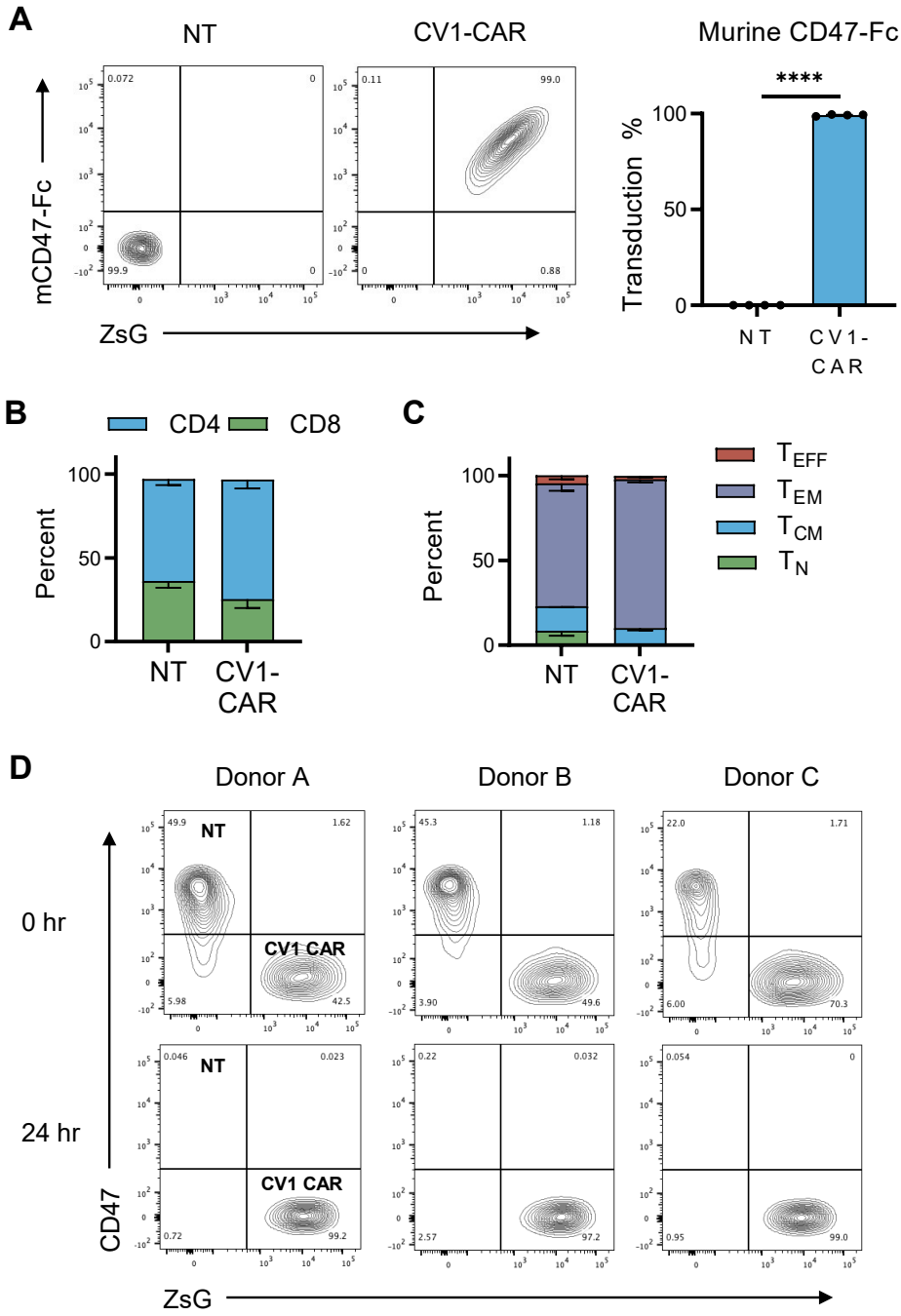
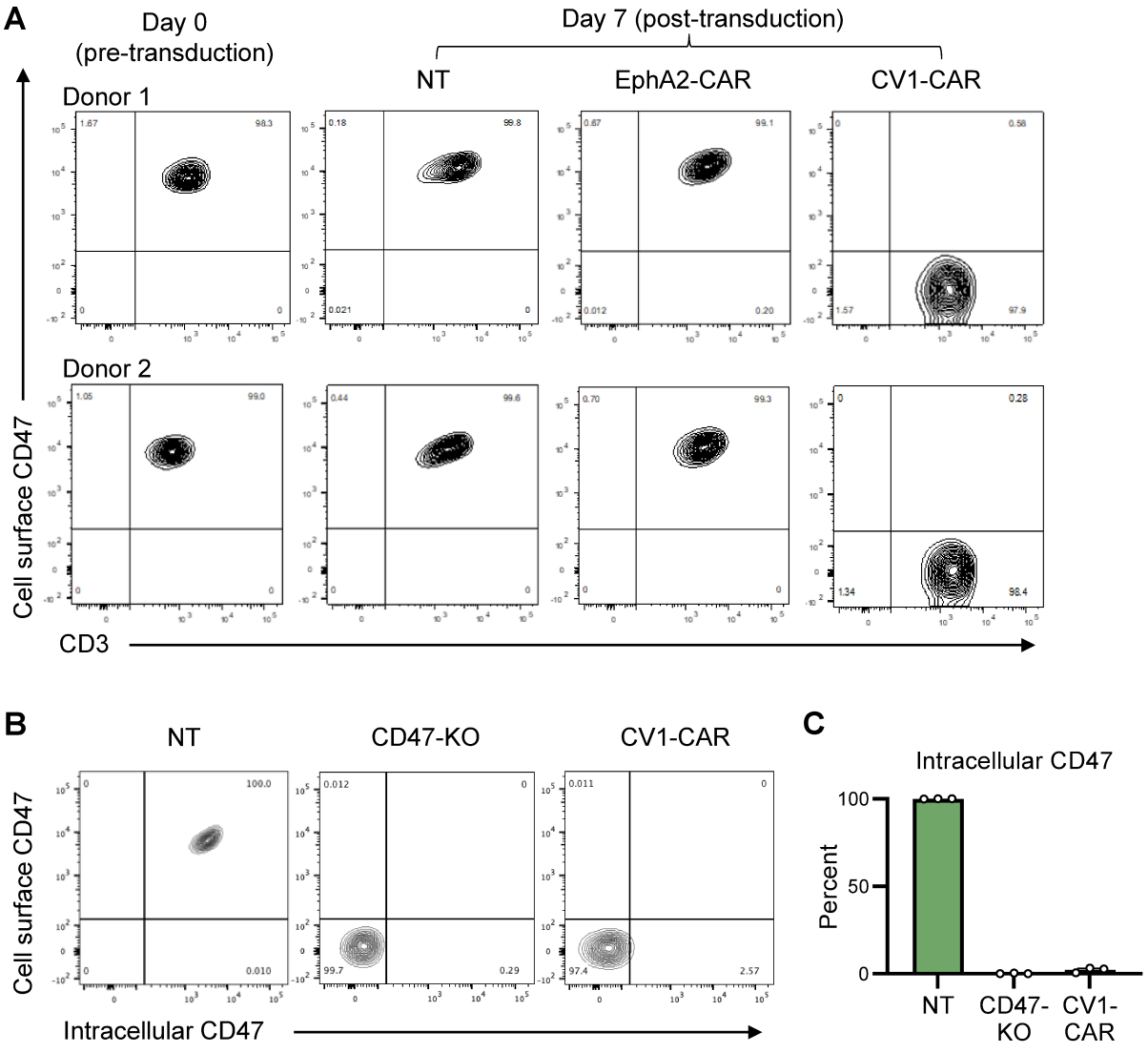
CV1	EEELQIIQPDKSVLVAAGETATLRCTITSLFPVGPIQWFRGAGPGRVLIY NQRQGPFPRVTTVSDTTKRNNMDFSIRIGNITPADAGTYYCIKFRKGS PDDVEFKSGAGTELSVRAKPS
CD8α Hinge/TM	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW APLAGTCGVLLLSLVITLYC
CD28 Costim	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
CD3ζ	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG LSTATKDTYDALHMQALPPR
P2A	*GSG*ATNFSLLKQAGDVEENPGP
ZsG	MAQSKHGLTKEMTMKYRMEGCVDGHKFVITGEGIGYPFKGKQAINLC VVEGGPLPFAEDILSAAFNYGNRVFTEYPQDIVDYFKNSCPAGYTWDR SFLFEDGAVCICNADITVSVEENCMYHESKFYGVNFPADGPVMKKMT DNWEPSCEKIIPVPKQGILKGDVSMYLLLKDGGRLRCQFDTVYKAKSV PRKMPDWHFIQHKLTREDRSDAKNQKWHLTEHAIASGSALP

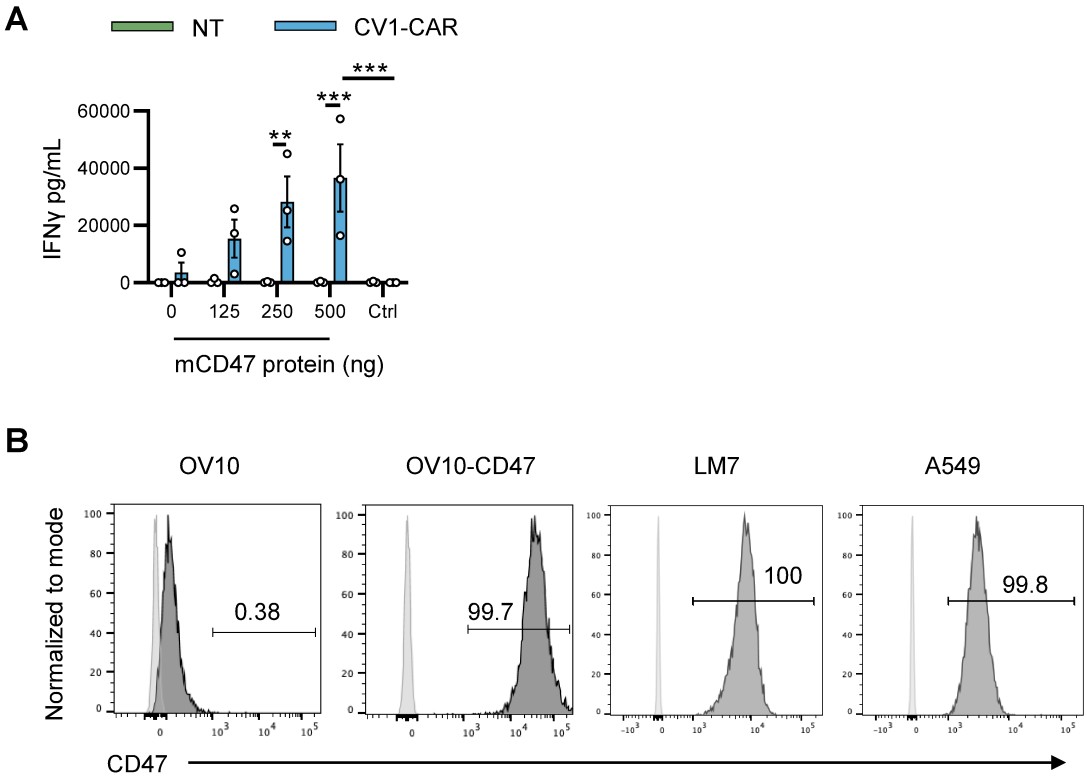
Supplemental figure 1. Amino acid sequences of CV1-CAR components. TM = transmembrane domain



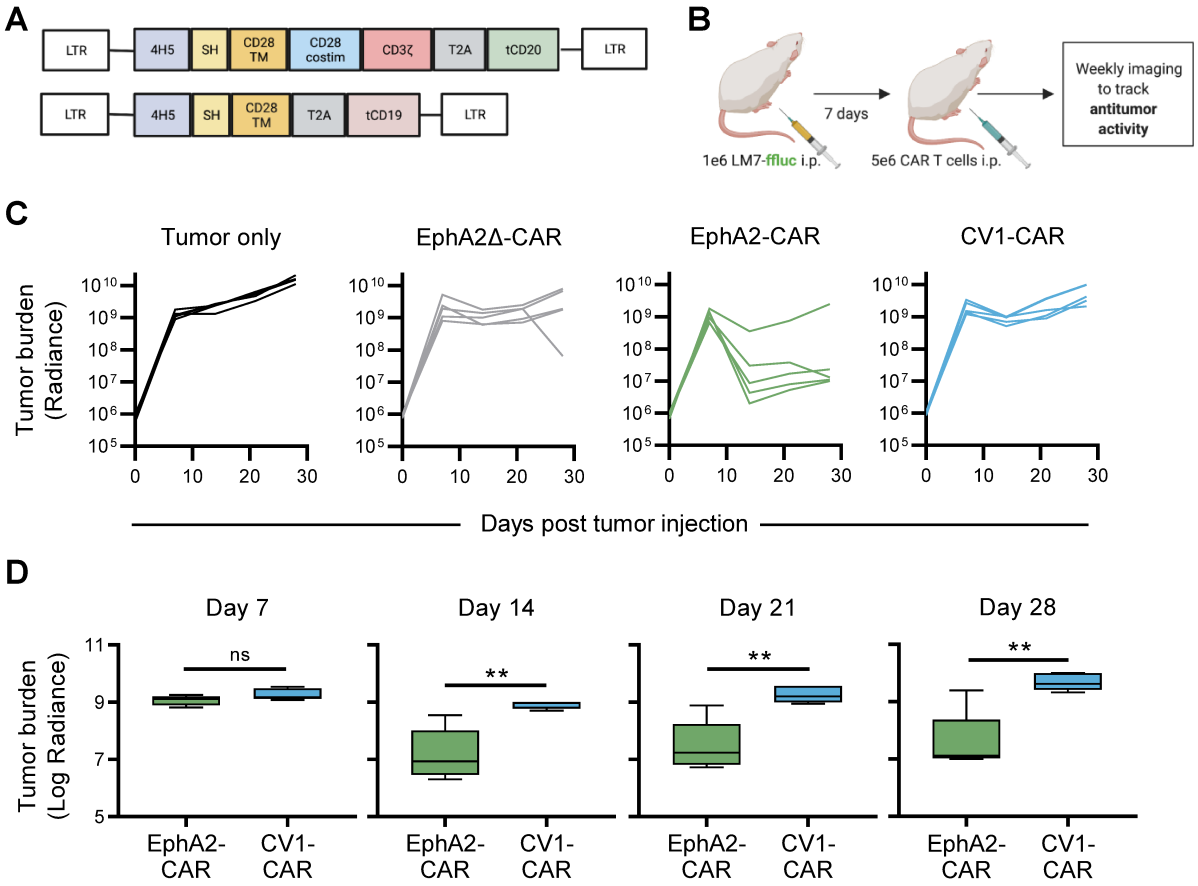
Supplemental figure 2. CV1-CAR T cells bind murine CD47, have phenotypic markers similar to NT T cells, and kill CD47-positive NT T cells. (**A**) Representative flow plots of NT or CV1-CAR T cells binding recombinant murine CD47-Fc protein (left panel), and summary data (right panel; n = 4). (**B**) CD4/CD8 ratios (n = 5) and (**C**) memory T cell phenotypes (n = 5) of NT and CV1-CAR T cells. T_{EFF} = terminal effector (CCR7-/CD45RO-), T_{EM} = effector memory (CCR7-/CD45RO+), T_{CM} = central memory (CCR7+/CD45RO+), T_N = naïve-like (CCR7+/CD45RO-). (**D**) CV1-CAR T cell killing of CD47-positive/ZsG-negative NT T cells was confirmed in 3 additional donors by flow cytometry immediately post coculture (0 hr) and 24 hr post coculture. Data represents mean ± SEM (A,B, and C). ****P < 0.0001 by paired t test (A).



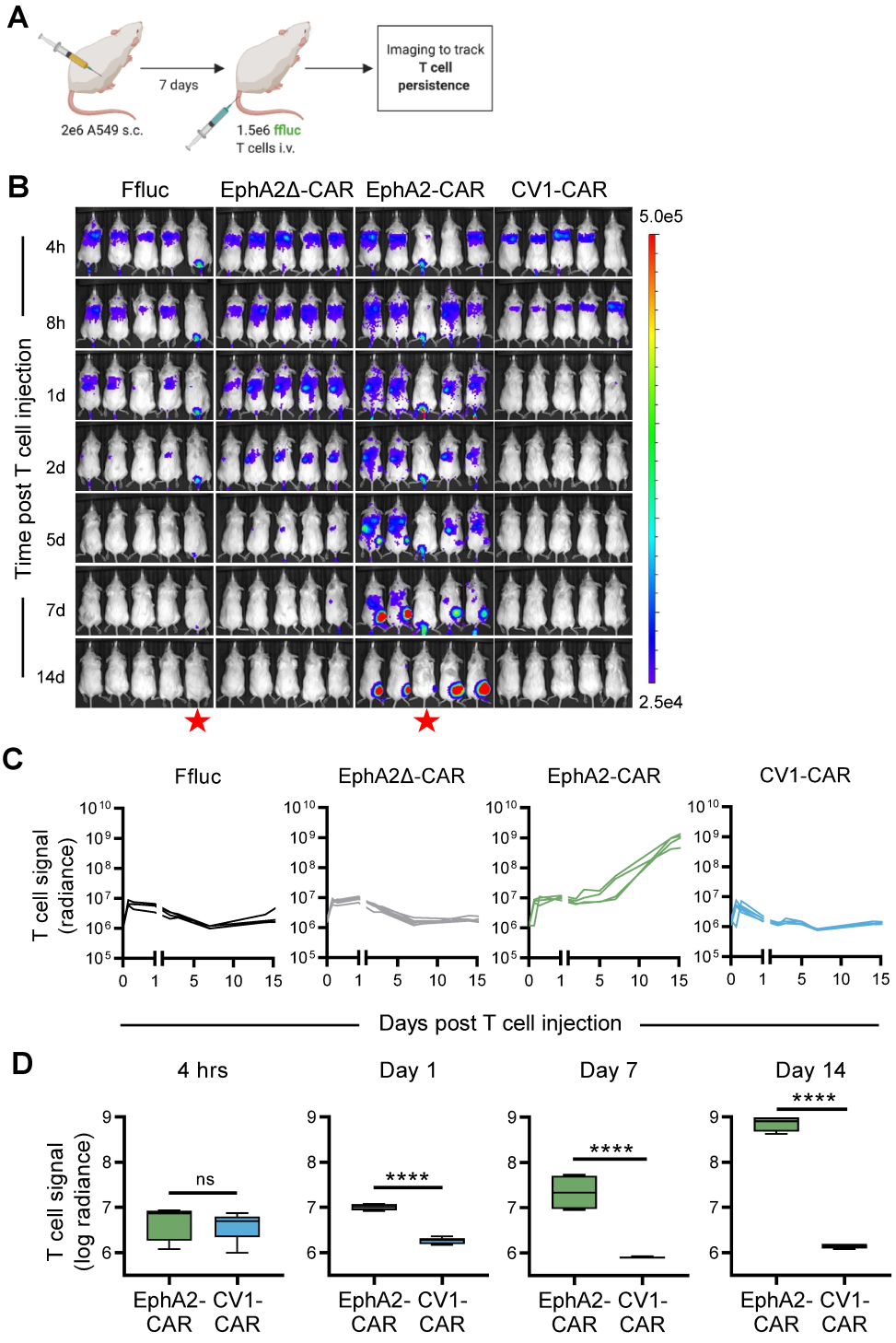
Supplemental figure 3. CV1-CAR T cells downregulate CD47 expression post-transduction. (**A**) Cell surface CD47 expression in CD3+ T cells pre-transduction (day 0), and 7 days post-transduction for NT, EphA2-CAR and CV1-CAR T cells. (**B**) Representative flow plots of intracellular and cell surface CD47 expression in NT, negative control CD47-knockout and CV1-CAR T cells. (**C**) Summary intracellular CD47 expression in NT, CD47-knockout and CV1-CAR T cells (n = 3). Data represents mean ± SEM (C).



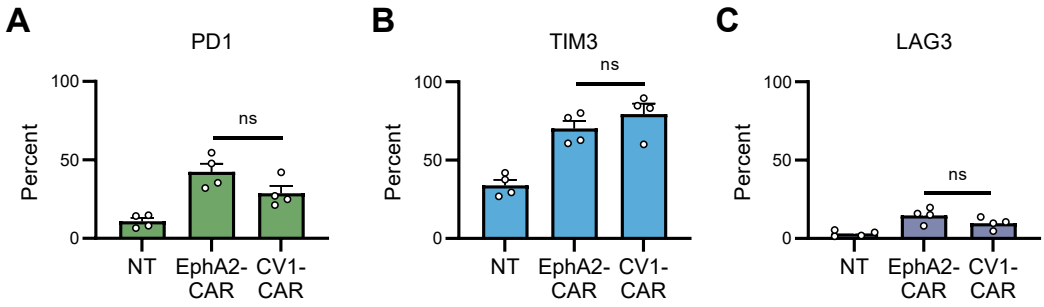
Supplemental figure 4. CV1-CAR T cells recognize murine CD47, and human cell lines express CD47. (**A**) CV1-CAR T cell IFNγ production was measured 24 hours post coculture with recombinant murine CD47 (mCD47) protein (n = 3). CV1-CAR T cells vs 0 ng protein and Ctrl protein (500 ng B7-H3 protein) represent the same data points as Fig. 2A because experiments were run concurrently with the same T cell donors. (**B**) CD47 expression on OV10 (CD47-negative) compared to OV10-CD47 (CD47-positive), LM7 (osteosarcoma) and A549 (lung adenocarcinoma). Data represents mean ± SEM (A). ***P* < 0.01, ****P* < 0.001, ns = non-significant by two-way ANOVA with Sidak's multiple comparisons test (A).



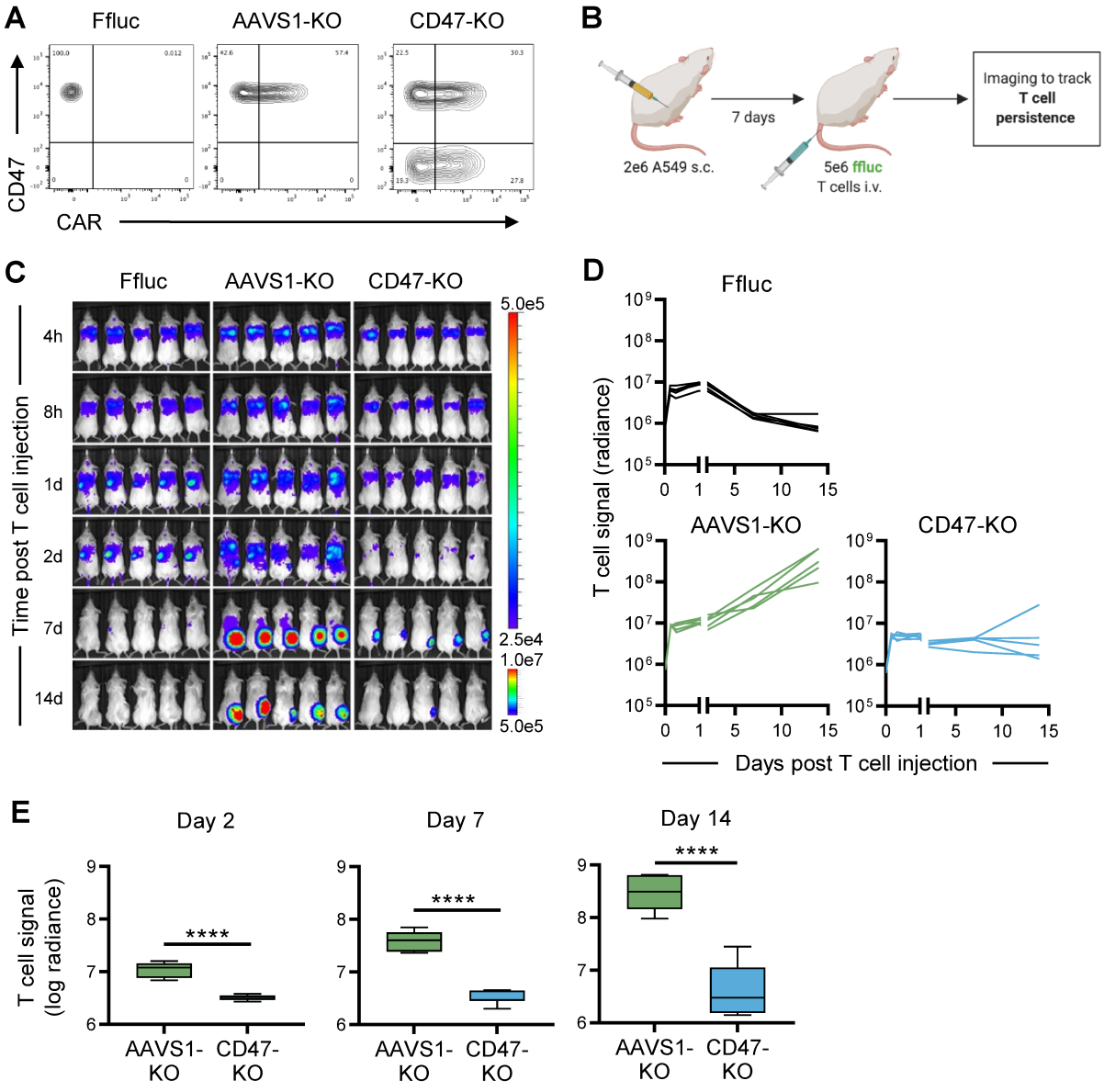
Supplemental figure 5. CV1-CAR T cells lack antitumor activity against LM7 at a high T cell dose. (**A**) Schematic representation of the EphA2-CAR with a truncated CD20 detection marker (top) and EphA2Δ-CAR (no costimulatory domain and no CD3ζ domain) with a truncated CD19 detection marker (bottom). SH = short hinge. (**B**) Schematic of the in vivo study. Mice were injected with 1 x 10^6 LM7.GFP.ffluc cells i.p. on day 0, followed by no treatment (tumor only) or 5 x 10^6 EphA2Δ-CAR, EphA2-CAR, or CV1-CAR T cells i.p. on day 7 (n = 5 per group). (**C**) Individual tumor bioluminescence measurements over time. (**D**) Summary tumor bioluminescence measurements over time. Radiance = photons/sec/cm2/sr. Data represents min to max (D). $^{**}P < 0.01$, ns = non-significant by unpaired t test (D).



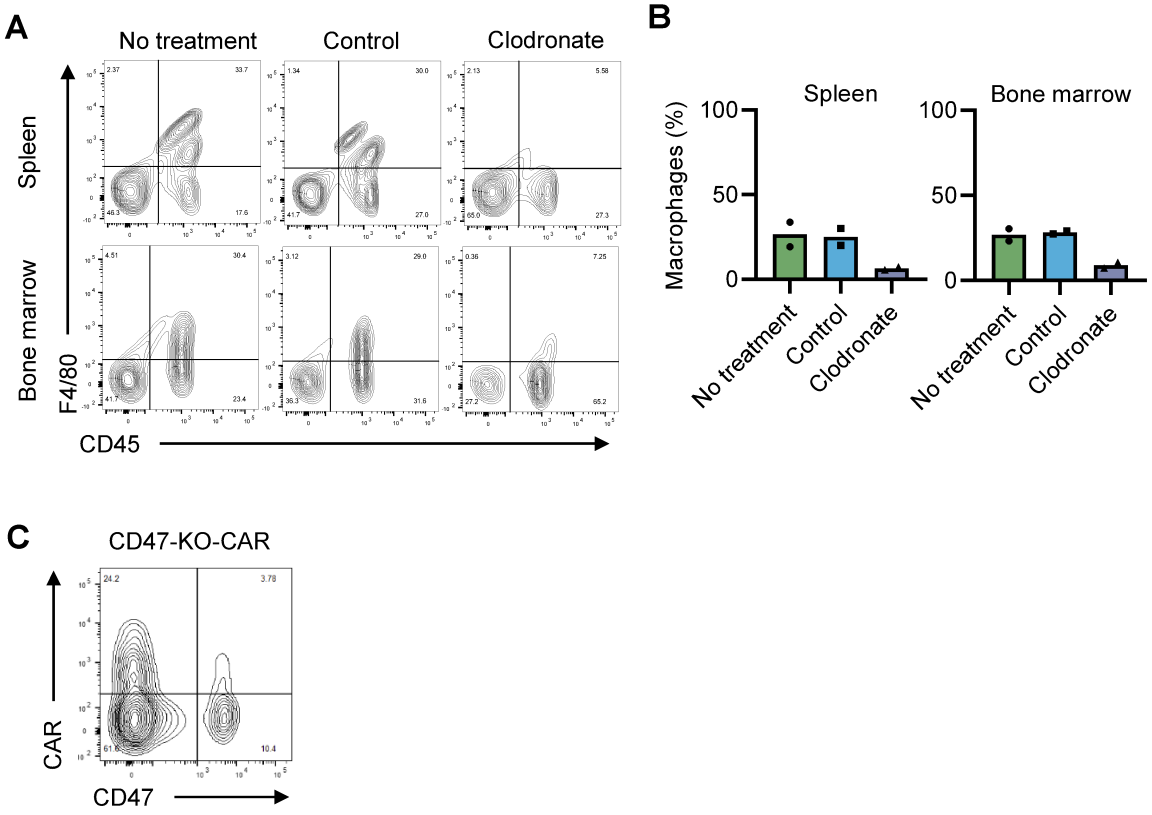
Supplemental figure 6. CV1-CAR T cells are detectable 4 to 8 hours post infusion and fail to persist *in vivo*. (**A**) Schematic of *in vivo* study. Mice were injected with 2 x 10^6 A549 cells s.c. on day 0, followed by 1.5 x 10^6 GFP.ffluc expressing EphA2Δ-CAR (n = 5), EphA2-CAR (n = 4), CV1-CAR (n = 5) or otherwise unmodified (Ffluc; n = 4) T cells i.v. on day 7. (**B**) Bioluminescence images at indicated time points post T cell injection (h = hour, d = day). (**C**) Individual bioluminescence measurements and (**D**) summary bioluminescence measurements over time. Red star indicates insufficient T cell injection and mouse not included in analysis. Radiance = photons/sec/cm2/sr. Data represents min to max (D). ****$P < 0.0001$, ns = non-significant by unpaired t test (D).



Supplemental figure 7. EphA2-CAR and CV1-CAR T cells have similar cell surface exhaustion markers post-transduction. Flow cytometry was performed to evaluate (**A**) PD1, (**B**) TIM3, and (**C**) LAG3 expression on non-transduced (NT; n = 4), EphA2-CAR (n = 4) and CV1-CAR (n = 4) T cells. Data represents mean ± SEM (A,B,C). ns = non-significant by one-way ANOVA with Dunnett's multiple comparisons test (A,B,C).



Supplemental figure 8. CD47-negative CAR T cell failure to persist in vivo is not due to the knockout procedure. CD47-negative CAR T cell persistence was evaluated in vivo using the A549 subcutaneous (s.c.) model and GFP.ffluc expressing CD47-KO EphA2-CAR (CD47-KO), control AAVS1-KO EphA2-CAR (AAVS1-KO), or otherwise unmodified (Ffluc) T cells. (**A**) CD47 and CAR surface expression post-transduction and knockout for indicated T cell products. (**B**) Schematic of the in vivo study. Mice were injected with 2 x 10^6 A549 cells s.c. on day 0, followed by 5 x 10^6 AAVS1-KO (n = 5), CD47-KO (n = 5), or Ffluc (n = 5) T cells i.v. on day 7. (**C**) Bioluminescence images for indicated time points post T cell injection (h = hour, d = day). (**D**) Individual and (**E**) summary bioluminescence measurements over time. Radiance = photons/sec/cm2/sr. Data represents min to max (E). ****$P < 0.0001$ by unpaired t test (E).



Supplemental figure 9. Clodronate depletes macrophages in the bone marrow and spleen of NSG mice. Flow cytometry was performed to detect macrophages in the spleen and bone marrow of NSG mice 48 hours after no treatment (n = 2) or treatment with control (n = 2) or clodronate (n = 2) liposomes. (**A**) Representative flow plots and (**B**) summary data for macrophage detection in the spleen and bone marrow. (**C**) EphA2-CAR and CD47 expression in CD47-KO-CAR T cells used in main **figure 6**.