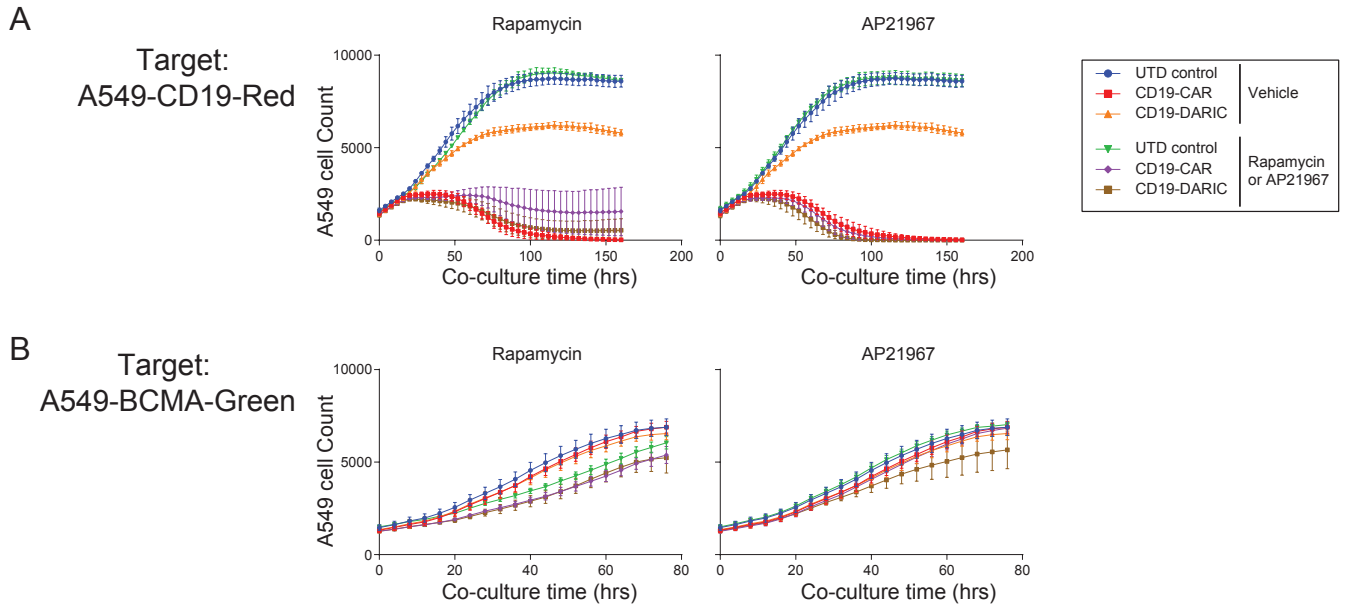


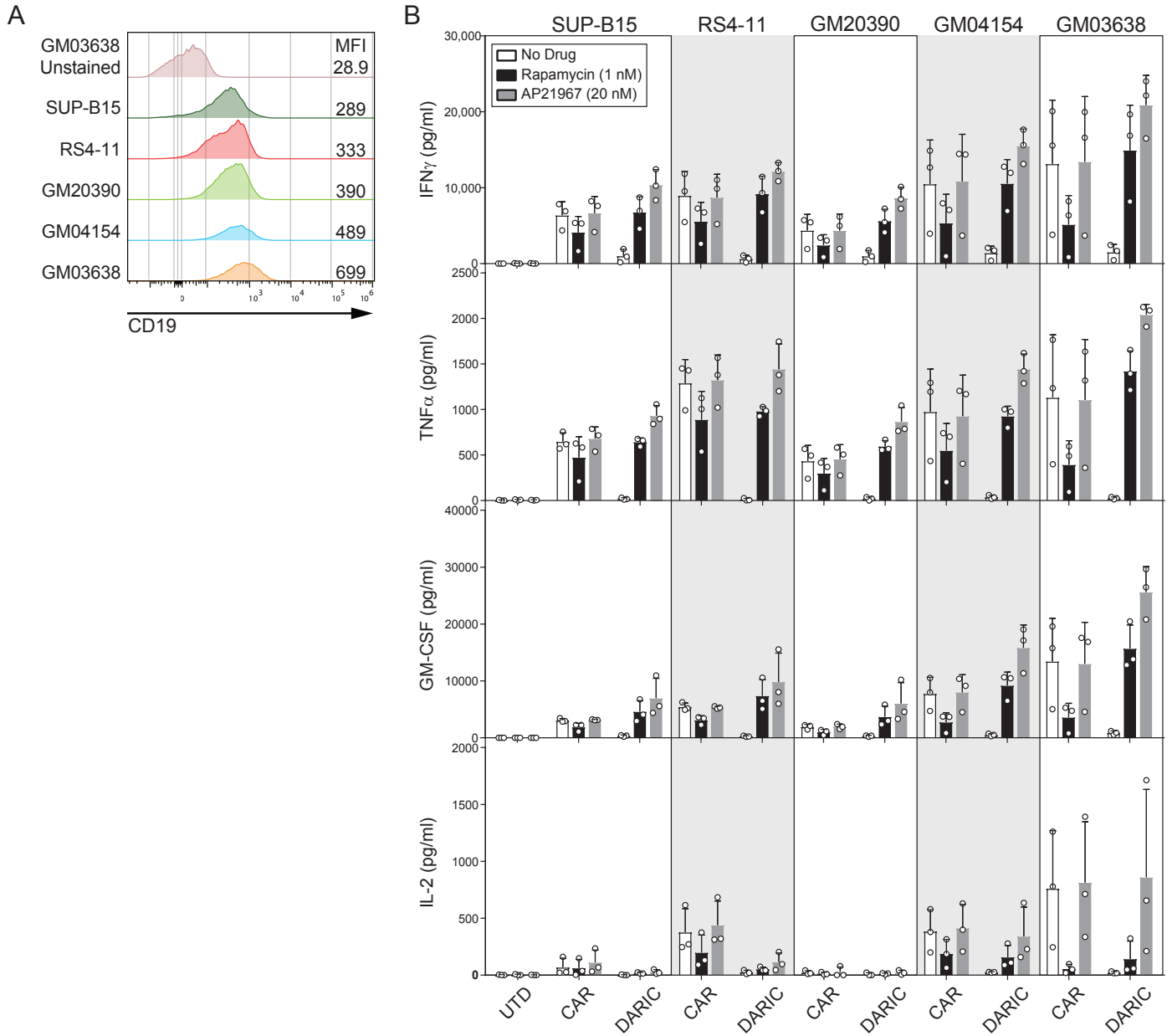
Supplemental Figure 1



Supplemental Figure 1: CD19-DARIC kill tumor cells with similar kinetics as CD19-CAR T cells

(A) The adherent tumor cell line A549 was transduced with CD19 and a red fluorescent reporter. The target cell was co-cultured with CD19-CAR or CD19-DARIC T cells at a 1:1 E:T ratio in the presence or absence of rapamycin (left) or AP21967 (right). Tumor cell elimination was tracked using an Incucyte live cell imager. (B) The CD19^{neg} A549 tumor cells were transduced with a green fluorescent reporter and cultured with CD19-CAR or CD19-DARIC T cells at a 1:1 E:T ratio in the presence or absence of rapamycin (left) or AP21967 (right).

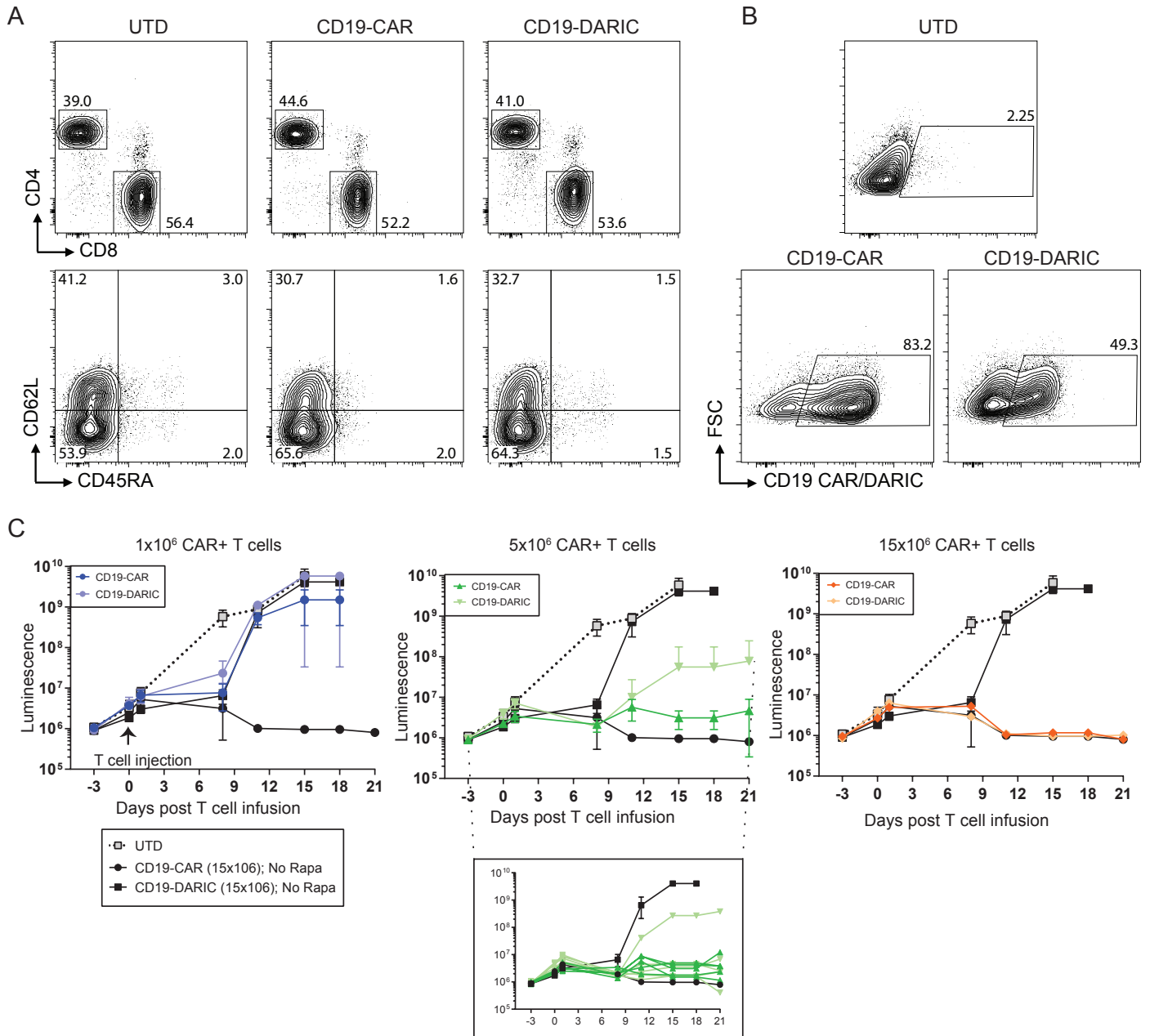
Supplemental Figure 2



Supplemental Figure 2: CD19-DARIC T cells target Acute Lymphoblastic Leukemia Cell Lines

(A) The expression of CD19 on different ALL-derived cell lines were analyzed by flow cytometry. The numbers on the right represent median fluorescent intensity (MFI) of CD19 expression. (B) The CD19-CAR and CD19-DARIC T cells were cultured at a 1:1 ratio with different ALL-derived cell lines in the presence or absence of rapamycin or AP21967. Supernatant was collected 24hr after culture initiation and cytokine levels were analyzed using 4-pex iQue QBead assay.

Supplemental Figure 3



Supplemental Figure 3: CD19 DARIC T cells control tumor in vivo at low cell numbers

(A) The phenotype and the CD4:CD8 composition of the T cells used for the in vivo dose titration was analyzed by flow cytometry. (B) The CD19-CAR and CD19-DARIC expression was analyzed using polyclonal antibody staining, as described in figure 1F. (C) NSG mice were injected with luminescent Nalm6 cells as described in Figure 5, and treated with various doses of CD19-CAR or CD19-DARIC+ T cells 10 days following tumor injection. Summary bioluminescence data is shown for each dose level. The UTD and the no drug CAR/DARIC groups are the same for all figures, while the “+ drug” groups are shown in different colors for each dose level. Error bars represent 5 mice. For the 5x10⁶ dose, individual bioluminescence traces for the CD19-CAR and CD19-DARIC mice are shown below the summary data.