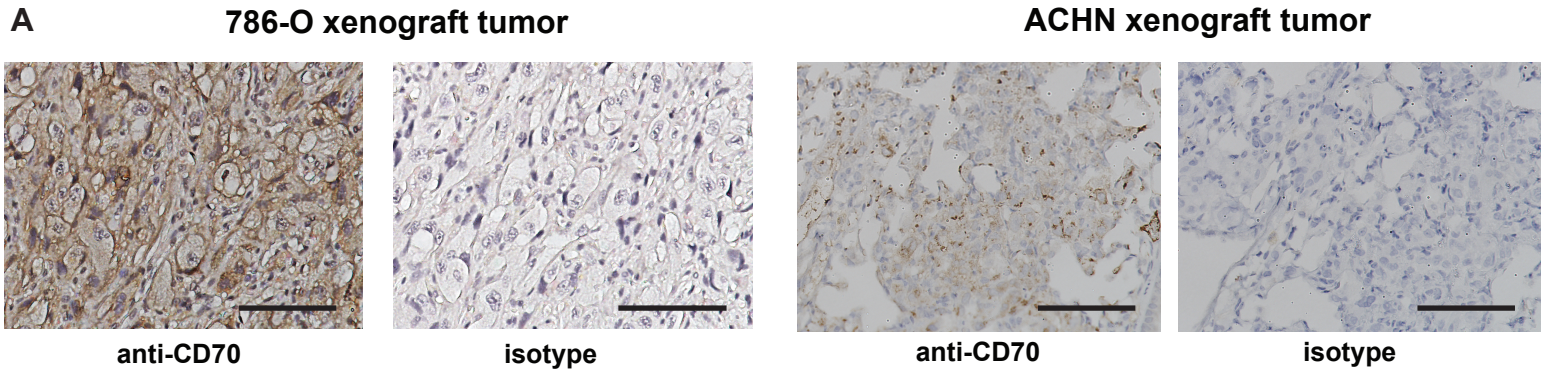
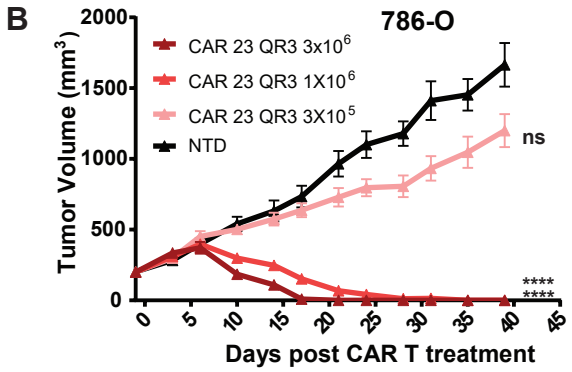
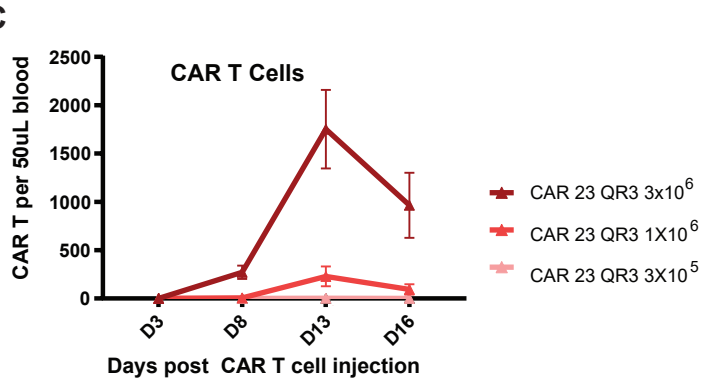
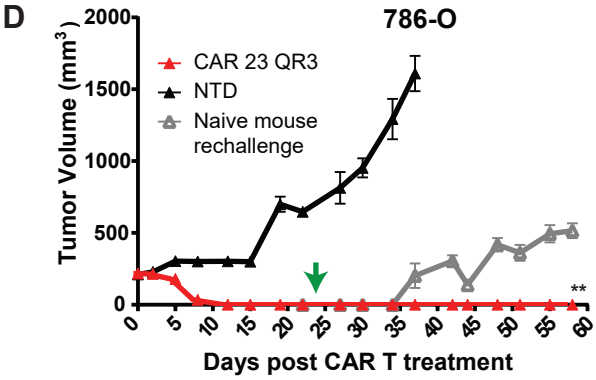
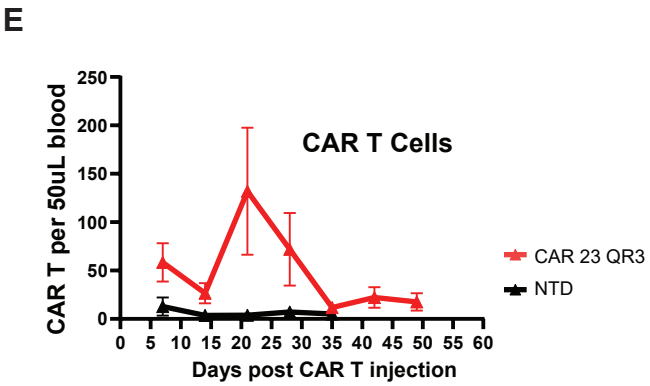
Fig. S7

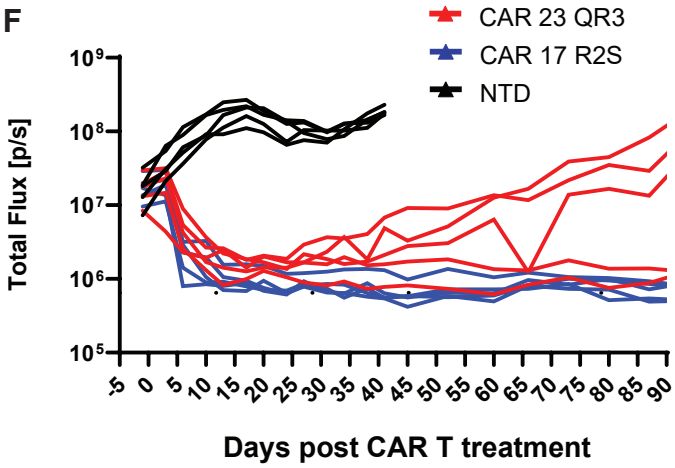

A
786-O xenograft tumor
ACHN xenograft tumor
anti-CD70
isotype
anti-CD70
isotype


B
786-O
CAR 23 QR3 3x10^6
CAR 23 QR3 1X10^6
CAR 23 QR3 3X10^5
NTD
ns
****
****
Tumor Volume (mm^3)
Days post CAR T treatment


C
CAR T Cells
CAR T per 50uL blood
D3
D8
D13
D16
Days post CAR T cell injection
CAR 23 QR3 3x10^6
CAR 23 QR3 1X10^6
CAR 23 QR3 3X10^5


D
786-O
CAR 23 QR3
NTD
Naive mouse rechallenge
**
Tumor Volume (mm^3)
Days post CAR T treatment


E
CAR T Cells
CAR T per 50uL blood
Days post CAR T injection
CAR 23 QR3
NTD


F
CAR 23 QR3
CAR 17 R2S
NTD
Total Flux [p/s]
Days post CAR T treatment

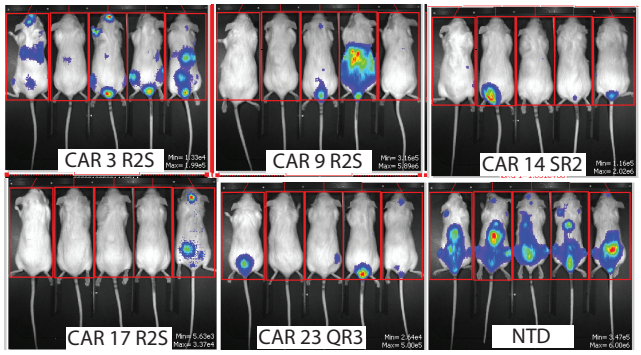

G
CAR 3 R2S
CAR 9 R2S
CAR 14 SR2
CAR 17 R2S
CAR 23 QR3
NTD

Fig. S7 continued

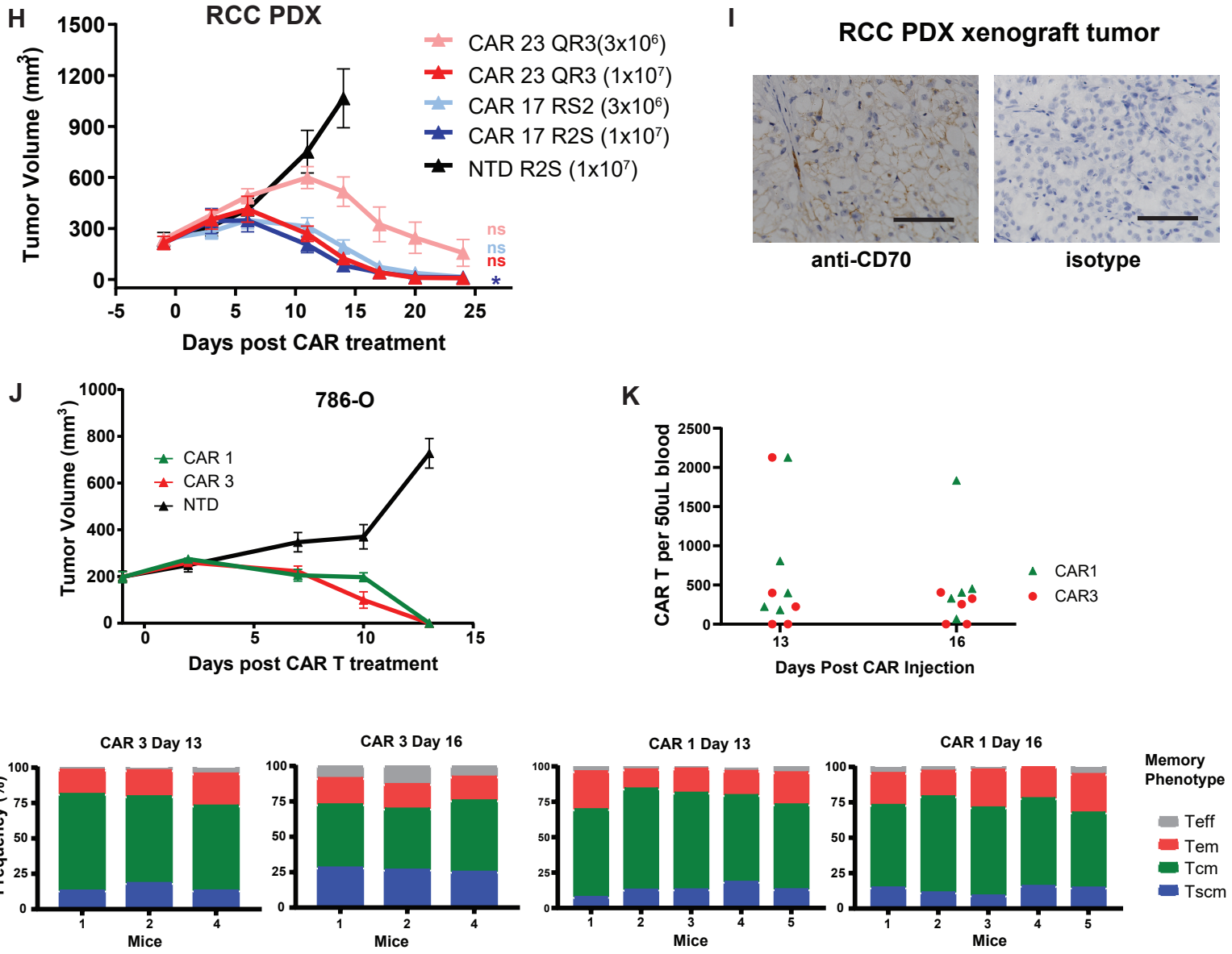


Figure S7. CD70 CAR T cells are highly efficacious and expand *in vivo*. **A,** 786-O and ACHN xenograft tumors were harvested from mice on day 34 and 30 respectively and stained for CD70 expression (brown). Scale bar, 50μm **B,** CD70 CAR 23 QR3 T cells injected at three doses displayed anti-tumor activity in a 786-O subcutaneous (SC) model. Tumor burden was measured by caliper twice weekly (n=10). **C,** Dose-dependent CAR 23 QR3 T cells expansion was observed, with a peak on day 13. CARs were enumerated in mouse blood by flow cytometry. **D,** CAR 23 QR3 T cells completely clear 786-O SC tumors at a dose of $3x10^6$. CAR-treated or naive mice were re-challenged with tumor on day 24 post CAR T injection (green arrow). Tumors grew in the naive mice, but were eliminated in the CAR treated animals. Tumor burden was measured by caliper twice weekly (n=10). **E,** CAR 23 QR3 T cells expanded and contracted following tumor clearance in a 786-O SC model (n=10). CAR T cell re-expansion was observed following a second SC tumor inoculation on day 24. CARs were enumerated in mouse blood by flow cytometry. **F,** CD70 CAR T cells demonstrate prolonged anti-tumor activity against an ACHN xenograft model. Study is the same as shown in Fig. 5G. Data is plotted for individual mice for two CARs followed for 90 days. **G,** Luminescent images of tumor burden (total flux) from the ACHN study shown in Fig. 5G, day 41 post CAR T infusion. **H,** CD70 CAR T cells with R-formats were highly active against an RCC PDX established xenograft model and induced complete tumor regression. Both CAR 17 (blue lines) and CAR 23 (red lines) completely eliminated tumors at the high dose of $10x10^6$ CAR^+ T cells/mouse. At the lower dose of $3x10^6$ CAR^+ T cells, both CARs elicited tumor regression but only CAR 17 was able to clear tumor to undetectable levels. Mice were implanted with RCC tumor fragments SC, and were randomized when tumors reached approximately $250mm^3$. CAR T cells were dosed IV at the indicated doses (n = 8) and tumor volumes monitored twice weekly. **I,** RCC PDX subcutaneous tumors were harvested and immunostained with anti-CD70 or isotype control (brown). Scale bar, 100μm. **J,** Both class 1 CAR 3 and class 2 CAR 1 eliminated SC 786-O tumors at a dose of $3x10^6$ CAR T cells per mouse (n=5). **K,** CAR T cells were observed in the blood of mice from the study shown in panel **J,** on day 13 and day 16 post CAR T injection as determined by flow cytometry. **L,** The memory subset phenotypes of CAR T cells from tumor bearing animals, shown in **E,** and **F,** were determined for class 1 CAR 3 and class 2 CAR 1 by flow cytometry using CD45RO and CD62L. Data for individual mice is shown. CAR T cells were present and analyzed in three mice for CAR 3 and five mice for CAR 1. For **B, H,** Statistics represent RMANOVA with Tukey's multiple comparison test, each group compared to corresponding NTD control (**** $p \leq 0.0001$, * $p \leq 0.05$, ns $p > 0.05$). For **D,** statistics represent paired T test (** $p \leq 0.0025$)