

Expanded View Figures

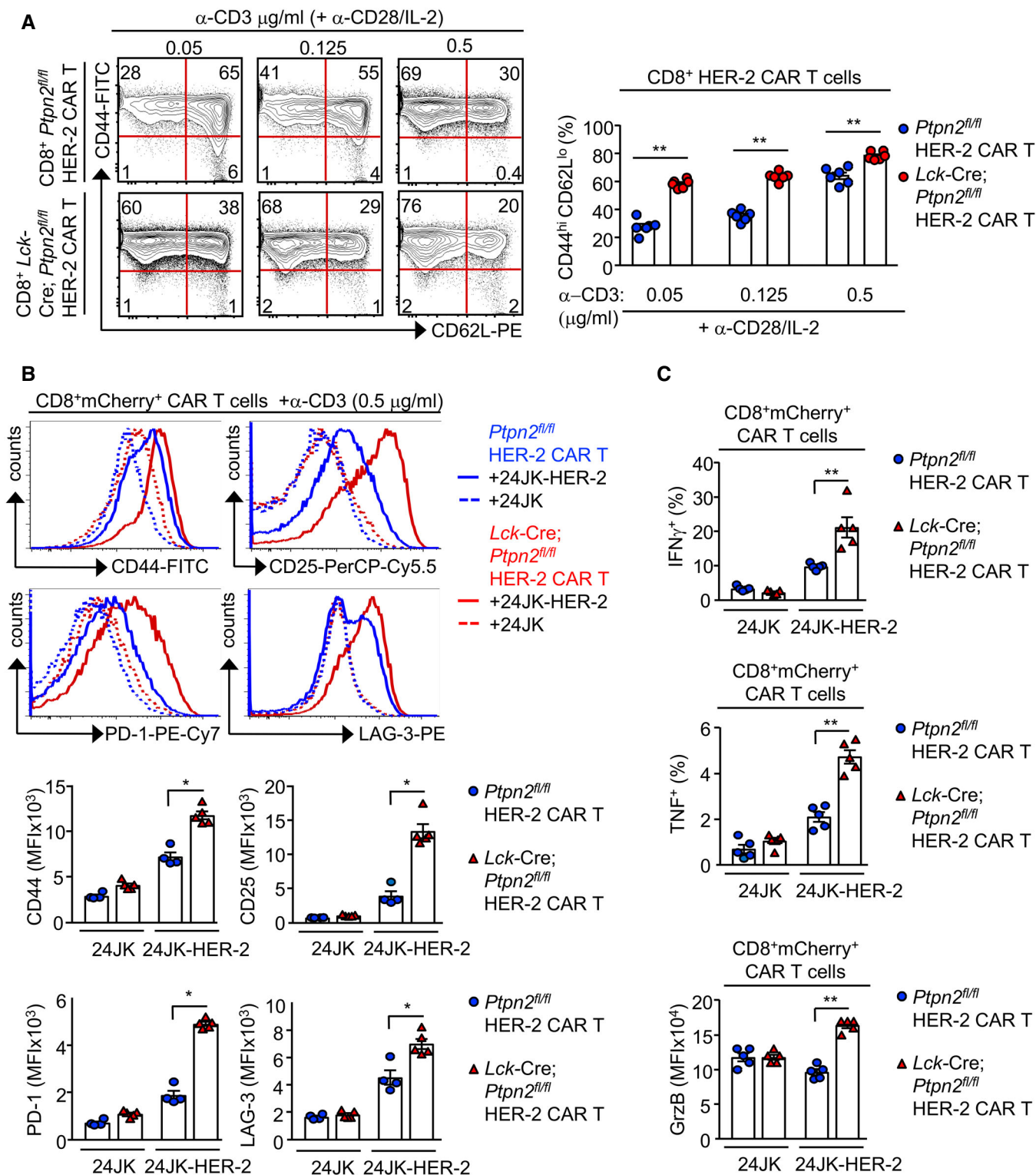


Figure EV1. PTPN2 deficiency enhances the generation and the antigen-induced activation of CAR T cells.

- A *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* HER-2 CAR T cells were generated with varying concentrations of α -CD3 (0.05, 0.125 and 0.5 μ g/ml) in the presence of α -CD28 (0.5 μ g/ml) and IL-2 (2 ng/ml). After 6 days in culture, the generation of effector/memory (CD44^{hi}CD62L^{lo}) CD8⁺ HER-2 CAR T cells was determined by flow cytometry.
- B, C *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* HER-2 CAR T cells were incubated with HER-2-expressing 24JK sarcoma cells (24JK-HER-2) and HER-2-negative 24JK sarcoma cells. (B) CD44, CD25, PD-1 and LAG-3 MFIs, (C) the proportion of CD8⁺IFN γ ⁺ versus CD8⁺TNF⁺ CAR T cells and GrzB mean fluorescence intensity (MFI) were determined by flow cytometry.

Data information: Significance in (A–C) was determined using 2-tailed Mann–Whitney *U*-test. **P* < 0.05, ***P* < 0.01.

Figure EV2. PTPN2 deficiency increases SFK and IL-2/15-induced STAT5 signalling in CD8⁺ HER-2 CAR T cells.

- A *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* HER-2 CD8⁺CD44^{hi}CD62L^{hi} or CD44^{hi}CD62L^{lo} CAR T cells were assessed for intracellular p(Y418)-SFK MFI by flow cytometry.
- B HER-2-specific *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* CAR T cells were incubated with plate-bound α -CD3 and CD25; CD122 and CD132 MFIs on CD8⁺CD44^{hi}CD62L^{hi} CAR T cells versus CD8⁺CD44^{hi}CD62L^{lo} CAR T cells were determined by flow cytometry.
- C, D *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* HER-2 CAR T cells were incubated with plate-bound α -CD3 and stimulated with recombinant (C) IL-2 and (D) IL-15 for the indicated time points. Intracellular p(Y694)-STAT-5 MFIs on CD8⁺CD44^{hi}CD62L^{hi} versus CD8⁺CD44^{hi}CD62L^{lo} CAR T cells were determined by flow cytometry.
- E *Ptpn2^{fl/fl}*, *Lck-Cre;Ptpn2^{fl/fl}* and *Lck-Cre;Ptpn2^{fl/fl};Lck^{+/-}* HER-2 CAR T cells were incubated with plate-bound α -CD3; CD122 and CD132 MFIs on CD8⁺CD44^{hi}CD62L^{lo} versus CD8⁺CD44^{hi}CD62L^{hi} CAR T cells were determined by flow cytometry.
- F *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl};Lck^{+/-}* HER-2 CAR T cells were incubated with plate-bound α -CD3 and stimulated with recombinant IL-2 and IL-15 for the indicated time points. Intracellular p(Y694)-STAT-5 MFIs on CD8⁺CD44^{hi}CD62L^{lo} CAR T cells were determined by flow cytometry.

Data information: Representative results (means $\pm$ SEM) are shown from two independent experiments. In (A, B, E), significance was determined using 2-tailed Mann–Whitney *U*-test. ***P* < 0.01.

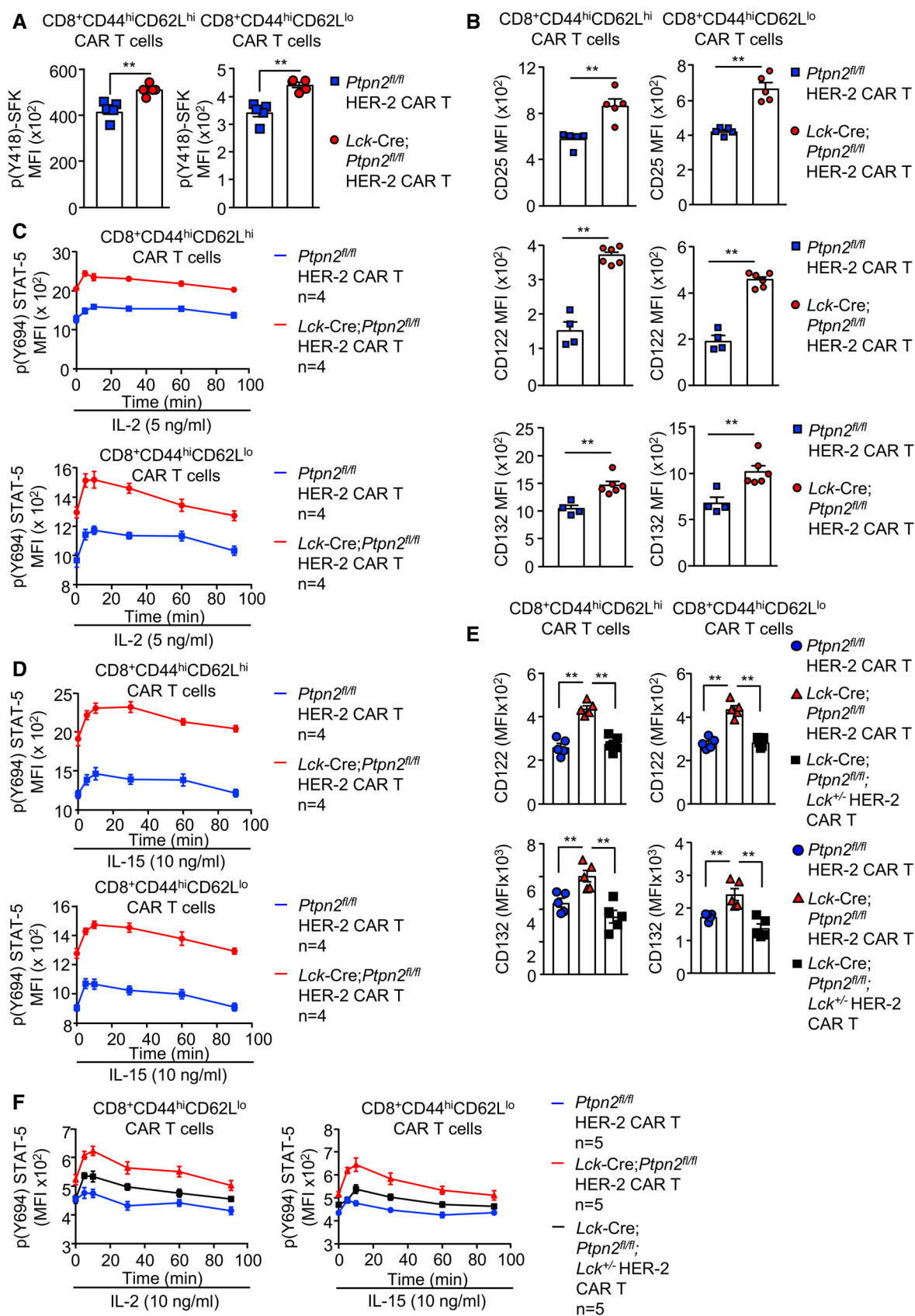


Figure EV2.

Figure EV3. PTPN2 deficiency enhances CAR T-cell proliferation and tumour infiltration.

- A CD8⁺CD44^{hi}CD62L^{hi} HER-2 CAR T cells generated from *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* splenocytes were stimulated with plate-bound α -CD3 and subsequently labelled with CTV and incubated with 24JK-HER-2 cells, and proliferation was determined by flow cytometry.
- B HER-2-E0771 tumours isolated from HER-2 TG mice on day 10 after adoptive *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* HER-2 CAR T-cell transfer were analysed for CD3⁺ T-cell infiltrates by immunohistochemistry. Scale bars: 500 μ m (full size) and 200 μ m (zoom).
- C HER-2-E0771 cells (2×10^5) were injected into the fourth inguinal mammary fat pads of female HER-2 transgenic (TG) mice. Six days after tumour injection, HER-2 TG mice received total body irradiation (4 Gy) followed by the adoptive transfer of 6×10^6 FACS-purified CD8⁺CD62L^{hi}CD44^{hi} central memory HER-2 CAR T cells generated from *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* splenocytes. Mice were injected with IL-2 (50,000 IU/day) on days 0–4 after adoptive CAR T-cell transfer. Lymphocytes were isolated from the tumours at day 16 post-adoptive transfer and CXCR3, CXCR5 and CCR7 MFIs on CD45⁺CD8⁺ T cells determined by flow cytometry.
- D, E Intracellular T-bet MFIs in CD8⁺ HER-2 CAR T cells were determined by flow cytometry.

Data information: Representative flow cytometry profiles and results (means $\pm$ SEM) are shown from two independent experiments. In (A, C, D, E), significance was determined using 2-tailed Mann–Whitney *U*-test. **P* < 0.05, ***P* < 0.01.

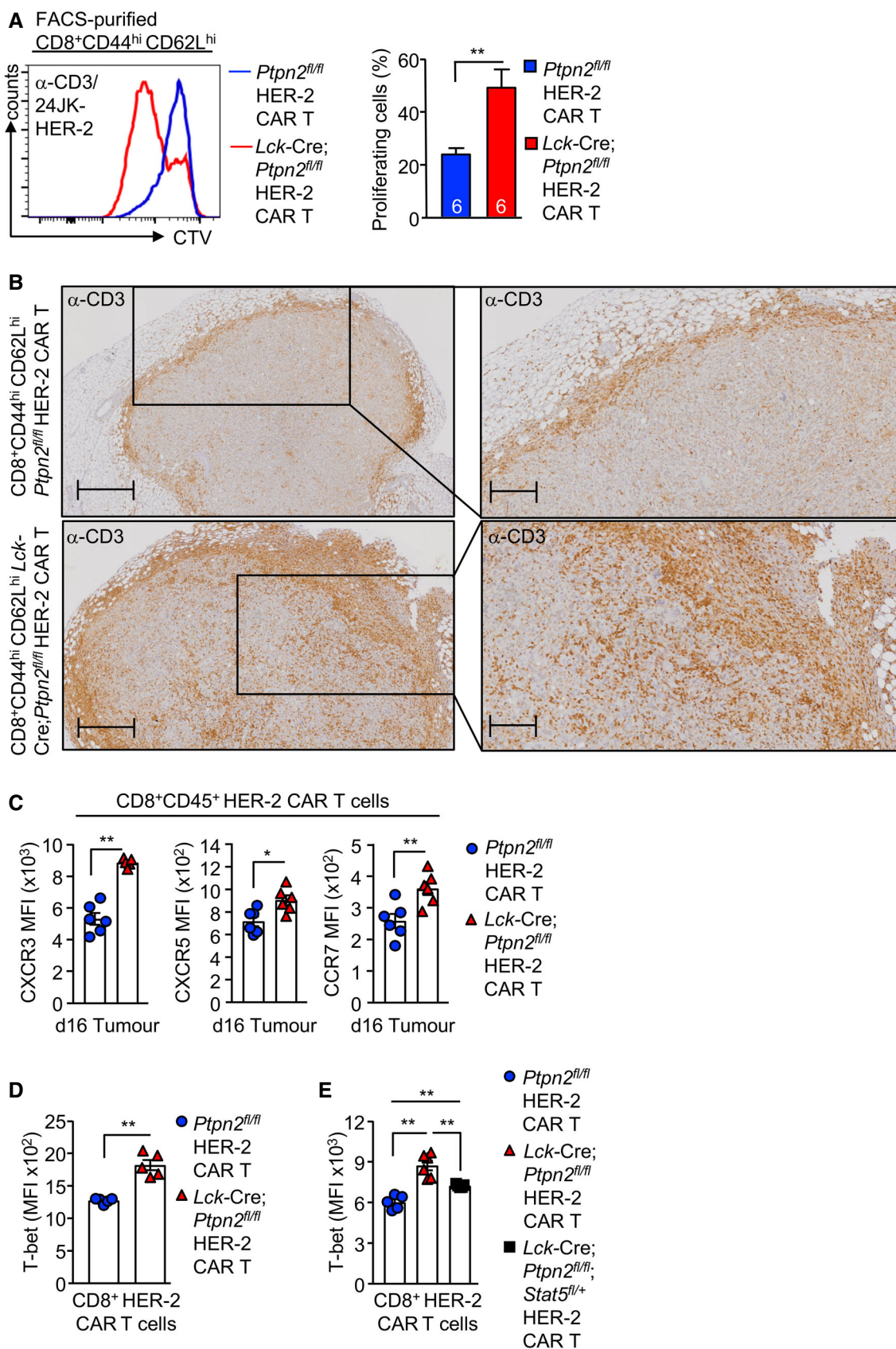


Figure EV3.

Figure EV4. PTPN2 deficiency in CAR T cells does not result in autoimmunity.

HER-2-E0771 mammary tumour cells (2×10^5) were injected into the fourth inguinal mammary fat pads of female HER-2 TG mice. Six days after tumour injection, HER-2 TG mice received total body irradiation (4 Gy) followed by the adoptive transfer of 20×10^6 FACS-purified CD8⁺CD44^{hi}CD62L^{hi} central memory HER-2 CAR T cells generated from *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* splenocytes. Mice were injected with IL-2 (50,000 IU/day) on days 0–4 after adoptive CAR T-cell transfer.

- A Serum cytokines were determined with the BD CBA Mouse Inflammation Kit[™].
- B, C Body core temperatures were measured using a mouse rectal probe, and (C) body weights were monitored.
- D Serum anti-nuclear antibodies (ANA) were measured using a mouse anti-nuclear antibodies Ig's (total IgA+G+M) ELISA Kit.
- E Serum AST and ALT activities were determined using a Transaminase II Kit.
- F, G Lamina propria CD45⁺CD8⁺ donor CAR T-cell numbers (F) and colon lengths (G) at 21 days post-adoptive CAR T-cell transfer.
- H Colons at 21 days post-CAR T-cell transfer were fixed in formalin and processed for histology (haematoxylin and eosin) monitoring for tissue architecture and lymphocytic infiltrates. Scale bars: 100 μ m.

Data information: Representative results (means $\pm$ SEM) are shown from two independent experiments. In (B, F), significance was determined using 2-tailed Mann–Whitney U-test. * $P < 0.05$, ** $P < 0.01$.

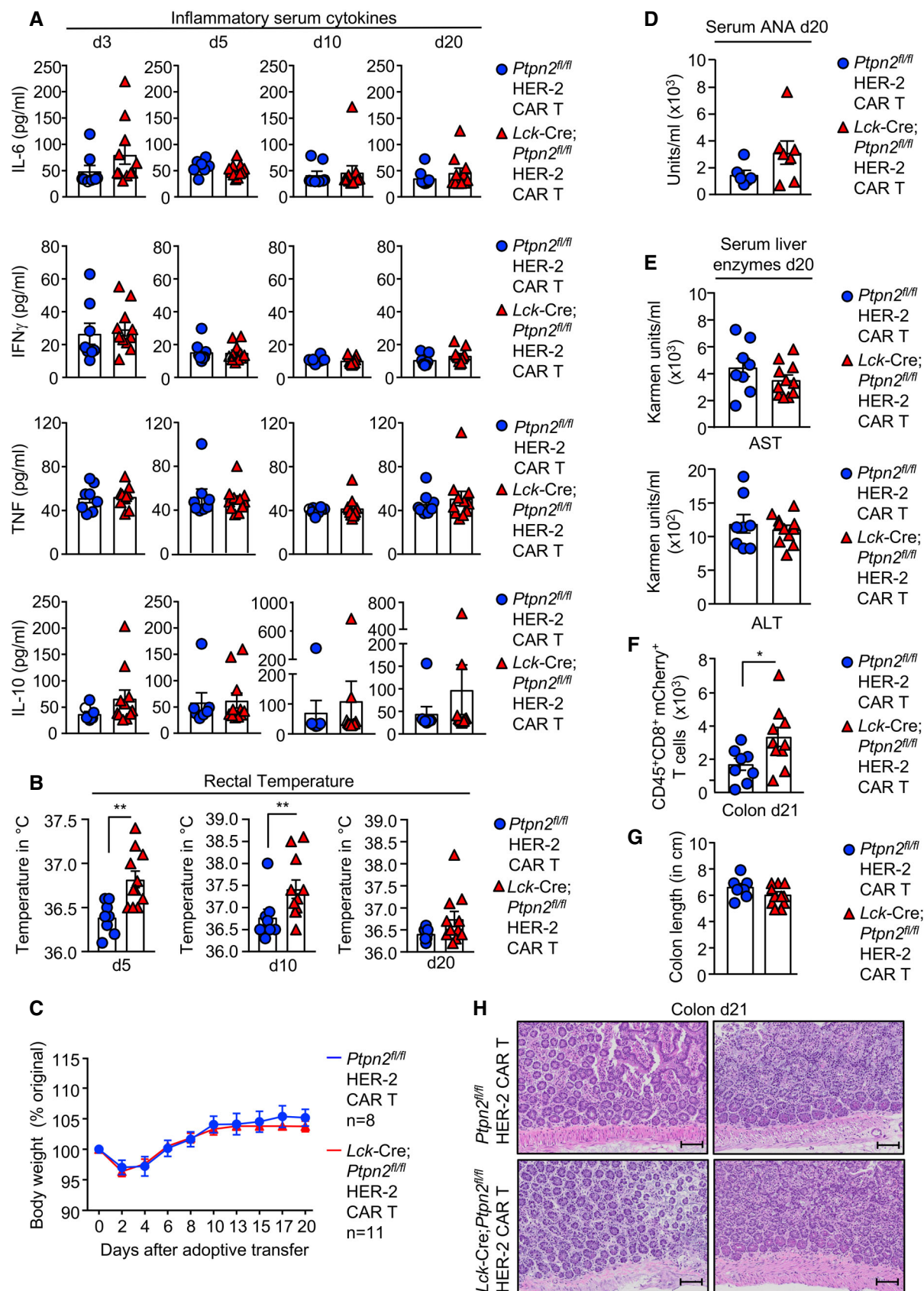


Figure EV4.

Figure EV5. Targeting Ptpn2 with siSTABLE™ siRNAs or using CRISPR-Cas9 RNP enhances tumour-specific CAR T-cell responses.

- A *Ptpn2^{fl/fl}* versus *Lck-Cre;Ptpn2^{fl/fl}* CD8⁺ HER-2 CAR T cells were treated with PTPN2-inhibitor (+) or vehicle (–) followed by incubation with 24JK-HER-2 versus 24JK sarcoma cells. The proportion of CD8⁺IFN γ ⁺ versus CD8⁺IFN γ ⁺TNF⁺ CAR T cells was determined by flow cytometry.
- B Human PBMCs were pretreated with PTPN2-inhibitor (+) or vehicle (–) and stimulated with α -CD3. MFIs for CD69 on CD8⁺CCR7⁺CD45RA⁺ T cells and CD8⁺CCR7⁺CD45RA⁺ T-cell numbers were determined by flow cytometry.
- C HER-2 CAR T cells transfected with GFP versus *Ptpn2* siSTABLE™ siRNAs were incubated with 24JK-HER-2 versus 24JK sarcoma cells. CD44, CD25, PD-1 and LAG-3 MFIs on CD8⁺ CAR T cells were determined by flow cytometry.
- D HER-2 CAR T cells transfected with GFP versus *Ptpn2* siSTABLE™ siRNAs were incubated with 24JK-HER-2 versus 24JK sarcoma cells. The proportion of CD8⁺IFN γ ⁺ CAR T cells was determined by flow cytometry.
- E HER-2 CAR T cells transfected with GFP versus *Ptpn2* siSTABLE™ siRNAs were incubated with 5 μ M CTV-labelled (CTV^{bright}) 24JK-HER-2 and 0.5 μ M CTV-labelled (CTV^{dim}) 24JK sarcoma cells. Antigen-specific target cell lysis (24JK-HER-2 versus 24JK response) was monitored for the depletion of CTV^{bright} 24JK-HER-2 cells by flow cytometry.
- F mCherry⁺ CAR T cells isolated from HER-2-E0771 tumours 21 days post-adoptive transfer were assessed for the proportion of CD8⁺IFN γ ⁺ versus CD8⁺IFN γ ⁺TNF⁺ CAR T cells by flow cytometry.
- G, H HER-2 CAR T cells were transfected with control versus *Ptpn2* sgRNAs plus Cas9 using the Lonza 4D-Nucleofector to delete PTPN2 by CRISPR-Cas9 RNP. (G) Control and PTPN2-deleted HER-2 CAR T cells were incubated with 24JK-HER-2 versus 24JK sarcoma cells and the proportion of CD8⁺IFN γ ⁺ CAR T cells determined by flow cytometry. (H) Alternatively, FACS-purified CD8⁺CD44^{hi}CD62L^{hi} CAR T cells and CD8⁺CD44^{hi}CD62L^{lo} CAR T control and PTPN2-deleted HER-2 CAR T cells were incubated with 5 μ M CTV-labelled (CTV^{bright}) 24JK-HER-2 cells and 0.5 μ M CTV-labelled (CTV^{dim}) 24JK sarcoma cells. Antigen-specific target cell lysis (24JK-HER-2 versus 24JK response) was assessed by monitoring for the depletion of CTV^{bright} 24JK-HER-2 cells by flow cytometry.

Data information: Representative results (means $\pm$ SEM) from at least two independent experiments are shown. In (A–D, F, G), significance was determined using 2-tailed Mann–Whitney *U*-test. In (E, H), significance was determined using 2-way ANOVA test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

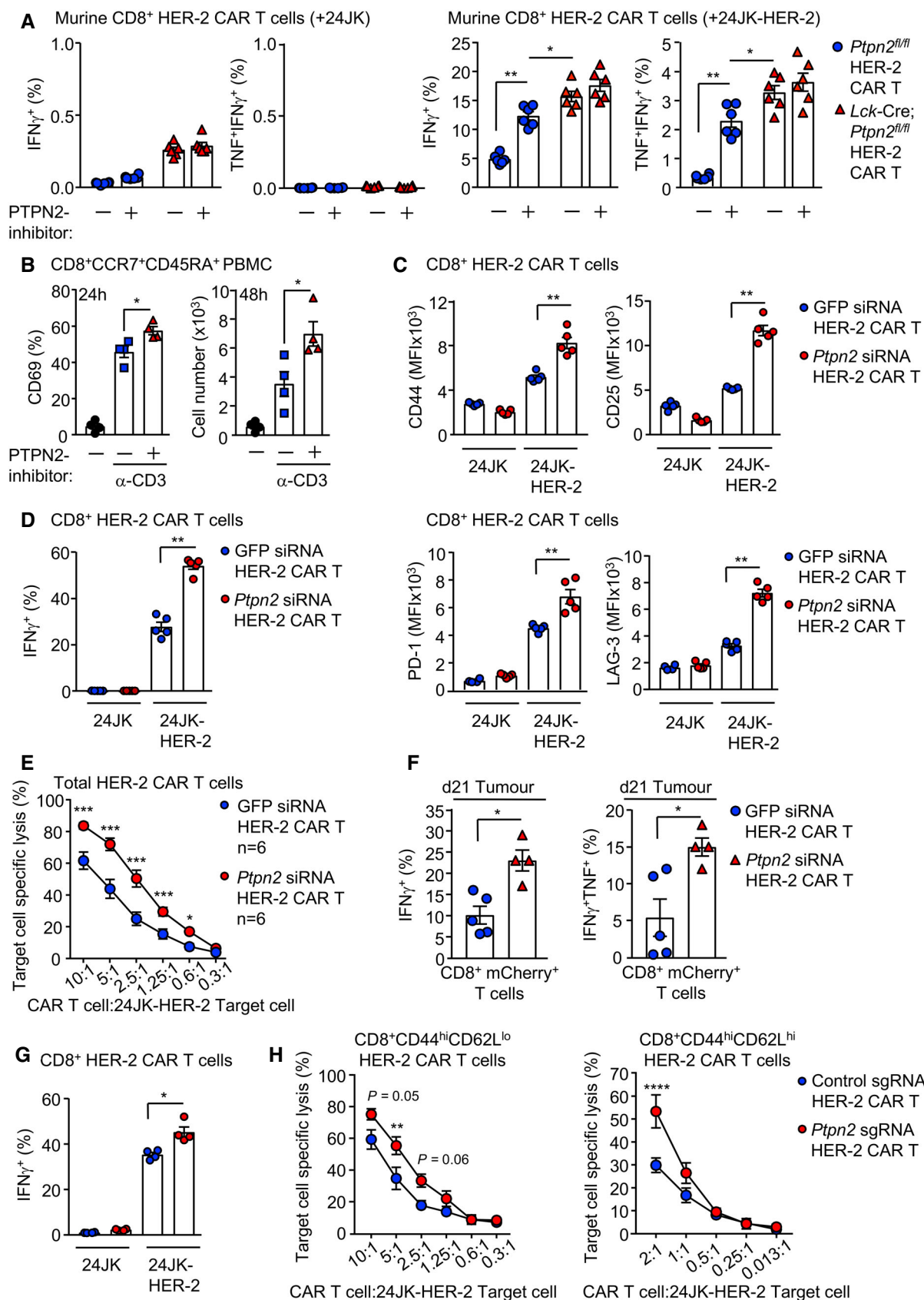


Figure EV5.