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## APPENDIX FIGURE LEGENDS

**Appendix figure S1. Tissue architecture and tumours in  $p53^{+/-}$  mice.** Gross images and histological analyses (Hematoxylin and Eosin: H&E) from 12 month old  $Ptpn2^{fl/fl};p53^{+/-}$  and  $Lck-Cre;Ptpn2^{fl/fl};p53^{+/-}$  mice. Scale bars H&E: Thymus/Thymoma/ Lymph node: 500  $\mu$ m (full size), 200  $\mu$ m and 50  $\mu$ m (zoom). Spleen: 800  $\mu$ m (full size), 200  $\mu$ m and 50  $\mu$ m (zoom). Sarcoma: 2 mm (full size), 300  $\mu$ m and 50  $\mu$ m (zoom). Scale bars organ images: 10 mm.

**Appendix figure S2. Inflammatory disease in  $Ptpn2^{fl/fl};p53^{+/-}$  and  $Lck-Cre;Ptpn2^{fl/fl};p53^{+/-}$  mice.** Inflammatory serum cytokines in **a)**  $Ptpn2^{fl/fl};p53^{+/-}$  versus  $Lck-Cre;Ptpn2^{fl/fl};p53^{+/-}$  mice and **b)**  $Ptpn2^{fl/fl}$  versus  $Lck-Cre;Ptpn2^{fl/fl}$  mice were determined with the LEGENDplex T<sub>H</sub> Cytokine Panel™ kit. **c)** Livers from 12 month old  $Ptpn2^{fl/fl};p53^{+/-}$  versus  $Lck-Cre;Ptpn2^{fl/fl};p53^{+/-}$  mice were fixed in formalin and processed for histology. Gross images are shown of  $Ptpn2^{fl/fl};p53^{+/-}$  livers bearing hepatomas (T). Scale bars H&E/Picrosirius Red: 3 mm (full size), 300  $\mu$ m and 100  $\mu$ m (zoom). Scale bars organ images: 5 mm. **d)** Day 26 (d26) tumour infiltrating lymphocytes (TILs) from  $Ptpn2^{fl/fl}$  versus  $Lck-Cre;Ptpn2^{fl/fl}$  mice were assessed for the proportion of CD4<sup>+</sup>CD25<sup>hi</sup>FoxP3<sup>+</sup> T<sub>regs</sub> by flow cytometry. **e)** Day 26 (d26) tumour infiltrating lymphocytes (TILs) from  $Ptpn2^{fl/fl}$  versus  $Lck-Cre;Ptpn2^{fl/fl}$  mice were incubated overnight with  $\alpha$ -CD3 (10  $\mu$ g/ml) and IL-2 (50 ng/ml) and intracellular Nur77 and IL-10 in CD4<sup>+</sup>CD25<sup>hi</sup>FoxP3<sup>+</sup> T<sub>regs</sub> were determined by flow cytometry. Significance in (b, d, e) was determined using 2-tailed Mann-Whitney U Test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

**Appendix figure S3. PTPN2 deficiency increases T cell infiltration and activation and decreases T cell exhaustion in a syngeneic model of triple negative breast cancer.** AT-3-OVA mammary tumour cells (1x10<sup>6</sup>) were injected into the fourth inguinal fat pads of female

Ly5.1<sup>+</sup> mice. Seven days after tumour injection FACS-purified naïve CD8<sup>+</sup>CD44<sup>lo</sup>CD62L<sup>hi</sup> lymph node T cells from Ly5.2<sup>+</sup> OT-1;*Ptpn2*<sup>fl/fl</sup> (2x10<sup>6</sup>) versus Ly5.2<sup>+</sup> OT-1;*Lck-Cre;Ptpn2*<sup>fl/fl</sup> (2x10<sup>6</sup>) mice were adoptively transferred into tumour-bearing Ly5.1<sup>+</sup> mice. **(a)** 21 days after adoptive transfer Ly5.1-Ly5.2<sup>+</sup> donor T cell numbers were determined in the draining lymph nodes (dLN) by flow cytometry. **(b)** 9 days after adoptive transfer Ly5.1-Ly5.2<sup>+</sup> donor T cell numbers were determined in the AT-3-OVA tumours by flow cytometry. **(c)** 9 days after adoptive transfer Ly5.1-Ly5.2<sup>+</sup> donor T cells isolated from mammary tumours or dLN were assessed for the proportion of Ly5.2<sup>+</sup>IFN $\gamma$ <sup>+</sup>TNF<sup>+</sup> and Ly5.2<sup>+</sup>GrzB<sup>+</sup> T cells. **(d-g)** 9 days or 21 days post adoptive transfer Ly5.1-Ly5.2<sup>+</sup> donor T cells isolated from mammary tumours or dLN were assessed for **(d, e, g)** PD-1 and LAG-3 MFIs or **(f)** CD44 MFIs. **(h)** AT-3-OVA tumour growth was monitored in female Ly5.1<sup>+</sup> mice up to 42 days post adoptive transfer. Representative flow cytometry profiles and results (means  $\pm$  SEM) from at least two independent experiments are shown. Significance in (a-c, e-g) was determined using 2-tailed Mann-Whitney U Test. In (h) significance was determined using 2-way ANOVA Test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001.

**Appendix figure S4. PTPN2 deficiency enhances the antigen-induced cytotoxic activity of CAR T cells.** **(a)** *Ptpn2*<sup>fl/fl</sup> versus *Lck-Cre;Ptpn2*<sup>fl/fl</sup> HER-2 CAR T cells were incubated with 24JK-HER-2 or 24JK sarcoma cells and the proportion of CD8<sup>+</sup>IFN $\gamma$ <sup>+</sup> CAR T cells was determined by flow cytometry. **(b)** FACS-purified CD8<sup>+</sup> central memory (CD44<sup>hi</sup>CD62L<sup>hi</sup>) and effector/memory (CD44<sup>hi</sup>CD62L<sup>lo</sup>) *Ptpn2*<sup>fl/fl</sup> versus *Lck-Cre;Ptpn2*<sup>fl/fl</sup> HER-2 CAR T cells were incubated with 5  $\mu$ M CTV-labelled (CTV<sup>bright</sup>) 24JK-HER-2 and 0.5  $\mu$ M CTV-labelled (CTV<sup>dim</sup>) 24JK sarcoma cells. Antigen-specific target cell lysis (24JK-HER-2 versus 24JK response) was monitored for the depletion of CTV<sup>bright</sup> 24JK-HER-2 cells by flow cytometry. Representative flow cytometry profiles and results (means  $\pm$  SEM) from at three independent

experiments are shown. Significance in (a) was determined using 2-tailed Mann-Whitney U Test. Significance in (b) was determined using 2-tailed Student's t test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

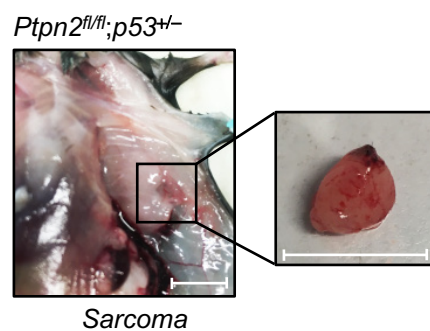
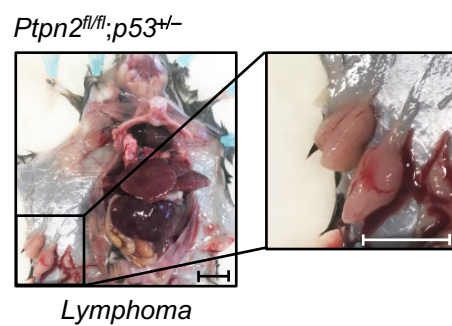
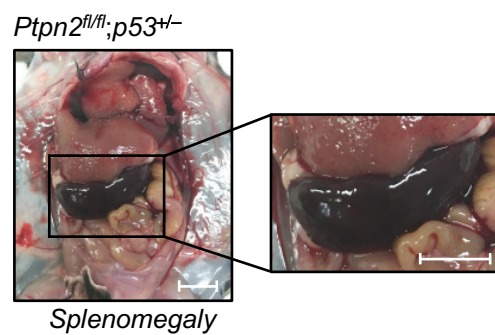
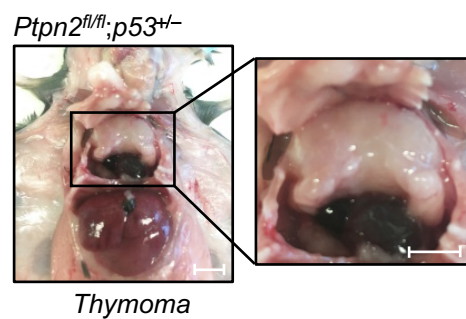
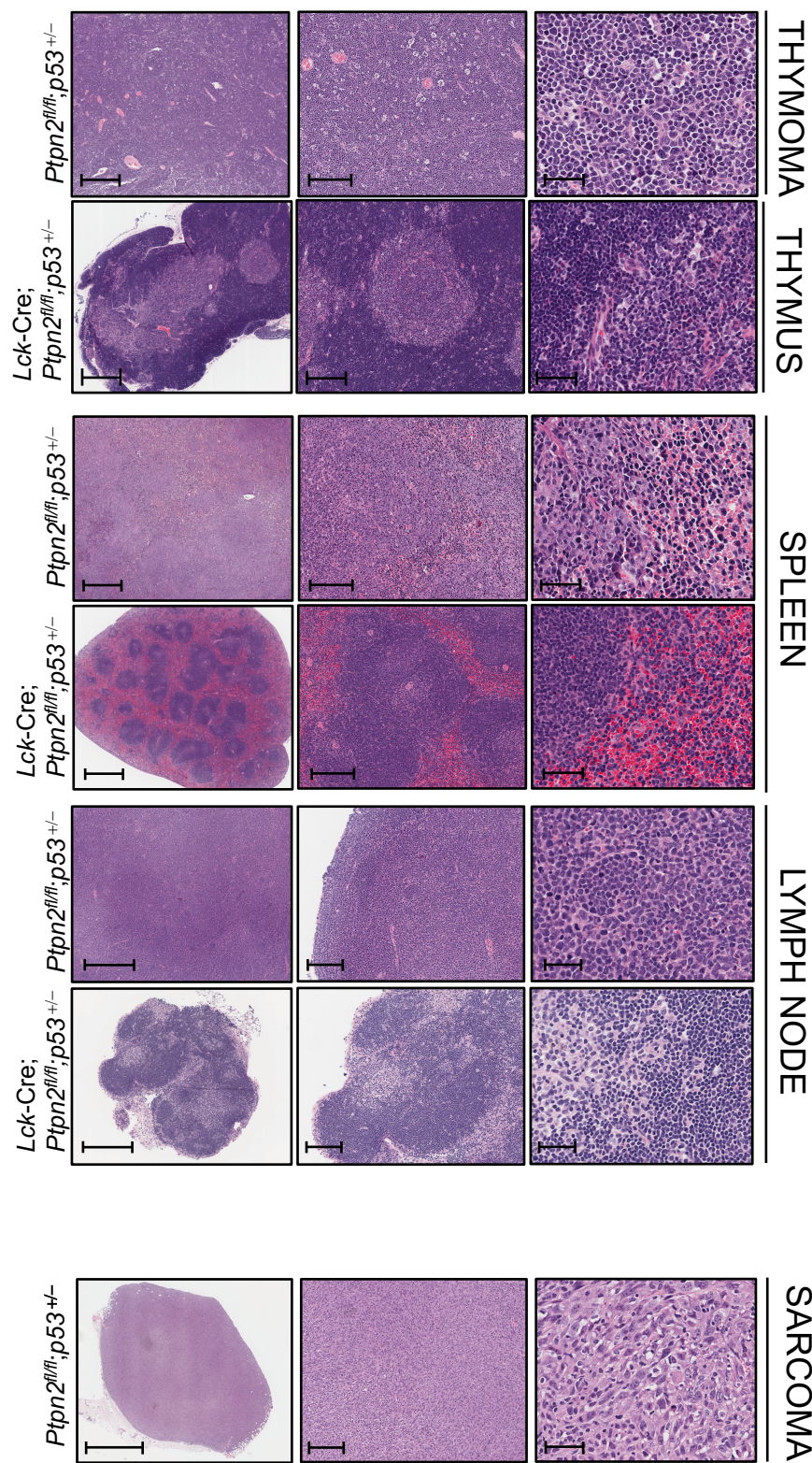
**Appendix figure S5. PTPN2 deficiency in CAR T cells does not result in systemic inflammation and collateral tissue damage.** HER-2-E0771 cells ( $2 \times 10^5$ ) were injected into the fourth inguinal mammary fat pads of female HER-2 TG mice. Six days after tumour injection HER-2 TG mice received total body irradiation (4 Gy) followed by the adoptive transfer of  $6 \times 10^6$  FACS-purified  $CD8^+CD44^{hi}CD62L^{hi}$  central memory HER-2 CAR T cells generated from *Ptpn2<sup>fl/fl</sup>* versus *Lck-Cre;Ptpn2<sup>fl/fl</sup>* splenocytes. Mice were injected with IL-2 (50,000 IU/day) on days 0-4 after adoptive CAR T cell transfer. **a)** Lymphocytes isolated from the spleens and livers of HER-2 TG recipient mice were assessed for  $CD3^+CD8^+$  donor CAR T cell numbers and PD-1 mean fluorescence intensities (MFI) by flow cytometry 21 days after adoptive CAR T cell transfer. **b)** Inflammatory serum cytokines in HER-2 TG recipient mice were determined with the LEGENDplex T<sub>H</sub> Cytokine Panel™ kit. **c)** The tumour-free contralateral fourth inguinal mammary fat pads were fixed in formalin at 21 days post CAR T cell transfer and processed for histological assessment (hematoxylin and eosin) monitoring for tissue architecture and lymphocytic infiltrates. Scale bars: 100  $\mu$ m. **d)** HER-2-E0771 breast cancer cells ( $2 \times 10^5$ ) 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  $20 \times 10^6$  FACS-purified  $CD8^+CD44^{hi}CD62L^{hi}$  central memory HER-2 CAR T cells generated from *Ptpn2<sup>fl/fl</sup>* versus *Lck-Cre;Ptpn2<sup>fl/fl</sup>* splenocytes. Mice were injected with IL-2 (50,000 IU/day) on days 0-4 after adoptive CAR T cell transfer and tumour growth was monitored. **e)** Lungs and livers were fixed in formalin at 21 days post CAR T cell transfer and processed for histological assessment (hematoxylin and eosin)



monitoring for tissue architecture and lymphocytic infiltrates. Scale bars: 200  $\mu$ m. Representative results (means  $\pm$  SEM) from at least two independent experiments are shown. In (d) significance was determined using 2-way ANOVA Test. \*\*\*\*p<0.0001.

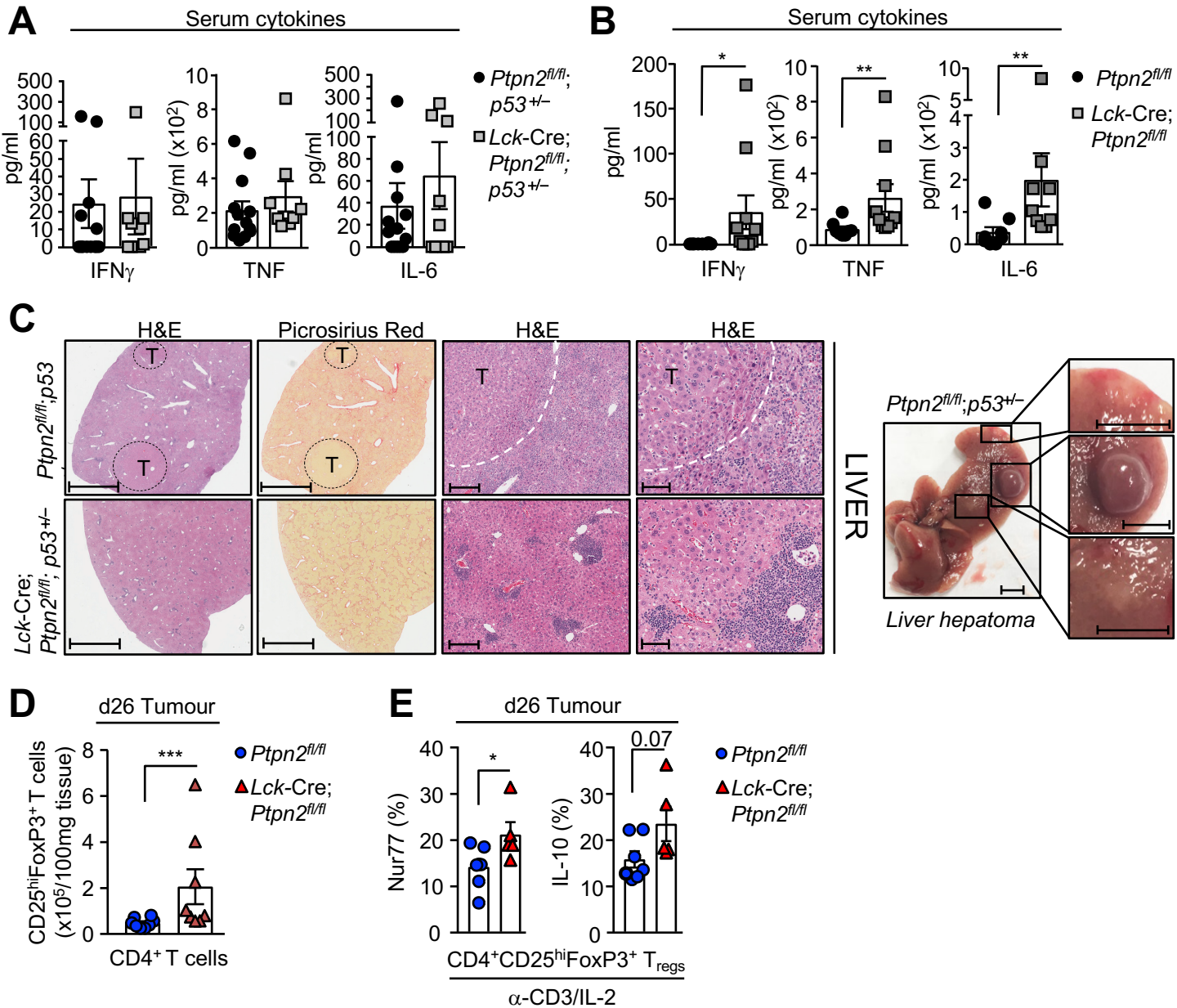
**Appendix figure S6. PTPN2 deficiency does not result in marked CAR T cell cerebellar infiltration and tissue damage.** a) Cerebella from +/+ C57BL/6 and HER-2 TG C57BL/6 mice were processed for  $\alpha$ -HER-2 immunohistochemistry. Scale bars: 2 mm. b) *HER-2* gene expression in cerebella from HER-2 TG mice, E0771-HER-2 tumours or HER-2-E0771 cells were assessed by quantitative real time PCR. c) Cerebella from HER-2 TG C57BL/6 mice administered *Ptpn2*<sup>fl/fl</sup> or *Lck-Cre;Ptpn2*<sup>fl/fl</sup> mCherry<sup>+</sup> HER-2 CAR T cells were processed for  $\alpha$ -CD3 $\epsilon$  immunohistochemistry 10 days after the transfer of HER-2 CAR T cells. CD3 $\epsilon$ <sup>+</sup>mCherry<sup>+</sup> CAR T cells were not detected in the cerebellar lobules, including the 8<sup>th</sup> cerebellar lobule (8Cb). Some CD3 $\epsilon$ <sup>+</sup>mCherry<sup>+</sup> staining was evident adjacent to the crus 1 of the ansiform lobule (Crus1) in mice treated with PTPN2-deficient HER-2 CAR T cells. Representative images for 3 mice per genotype are shown. d) Cerebella from HER-2 TG mice administered *Ptpn2*<sup>fl/fl</sup> or *Lck-Cre;Ptpn2*<sup>fl/fl</sup> HER-2 CAR T cells were processed for histology (hematoxylin and eosin: H&E) 10 days after the adoptive transfer of HER-2 CAR T cells to assess gross tissue architecture. e) CD44 MFIs on CD45<sup>+</sup>CD8<sup>+</sup>CD3<sup>+</sup> mCherry<sup>+</sup> CAR T cells and the proportion of IFN $\gamma$ <sup>+</sup>TNF<sup>+</sup> versus GrzB<sup>+</sup> CD45<sup>+</sup>CD8<sup>+</sup>CD3<sup>+</sup> mCherry<sup>+</sup> CAR T cells isolated from HER-2-E0771 tumours 10 days post adoptive CAR T cell transfer were determined by flow cytometry. f) HER-2 TG mice administered *Lck-Cre;Ptpn2*<sup>fl/fl</sup> HER-2 CAR T cells were subjected to a rotarod test 50 days post tumour clearance and the latency to fall determined in consecutive trials. g) *Cxcl9* and *Cxcl10* gene expression in cerebella from C57BL/6 and HER-2 TG mice and E0771-HER-2 tumours were assessed by quantitative real time PCR. Representative results (means  $\pm$  SEM) from at least two independent experiments

are shown. In (b, g) significance was determined using 1-way ANOVA Test. In (e) significance was determined using 2-tailed Mann-Whitney U Test. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

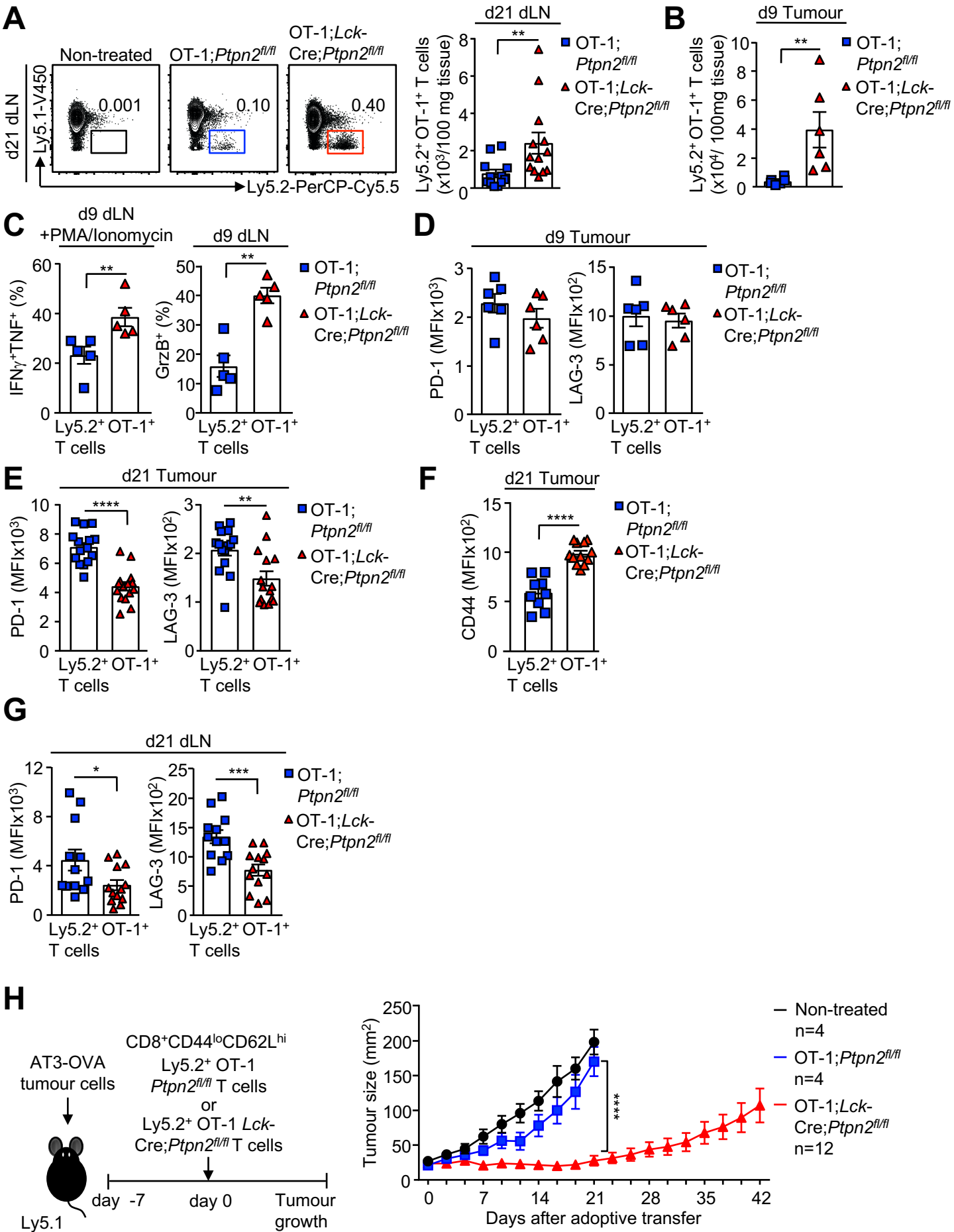


Appendix Figure S1

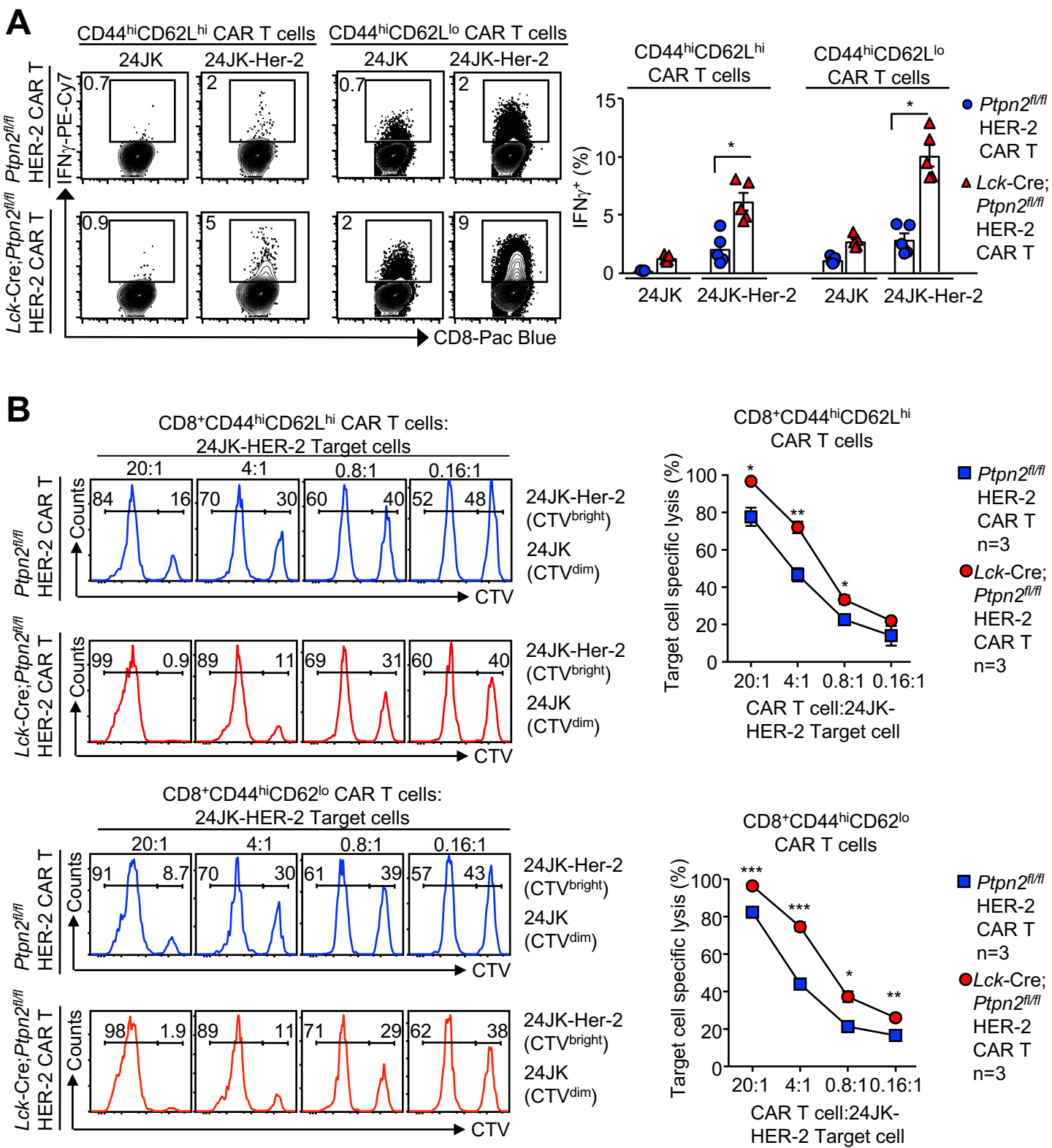




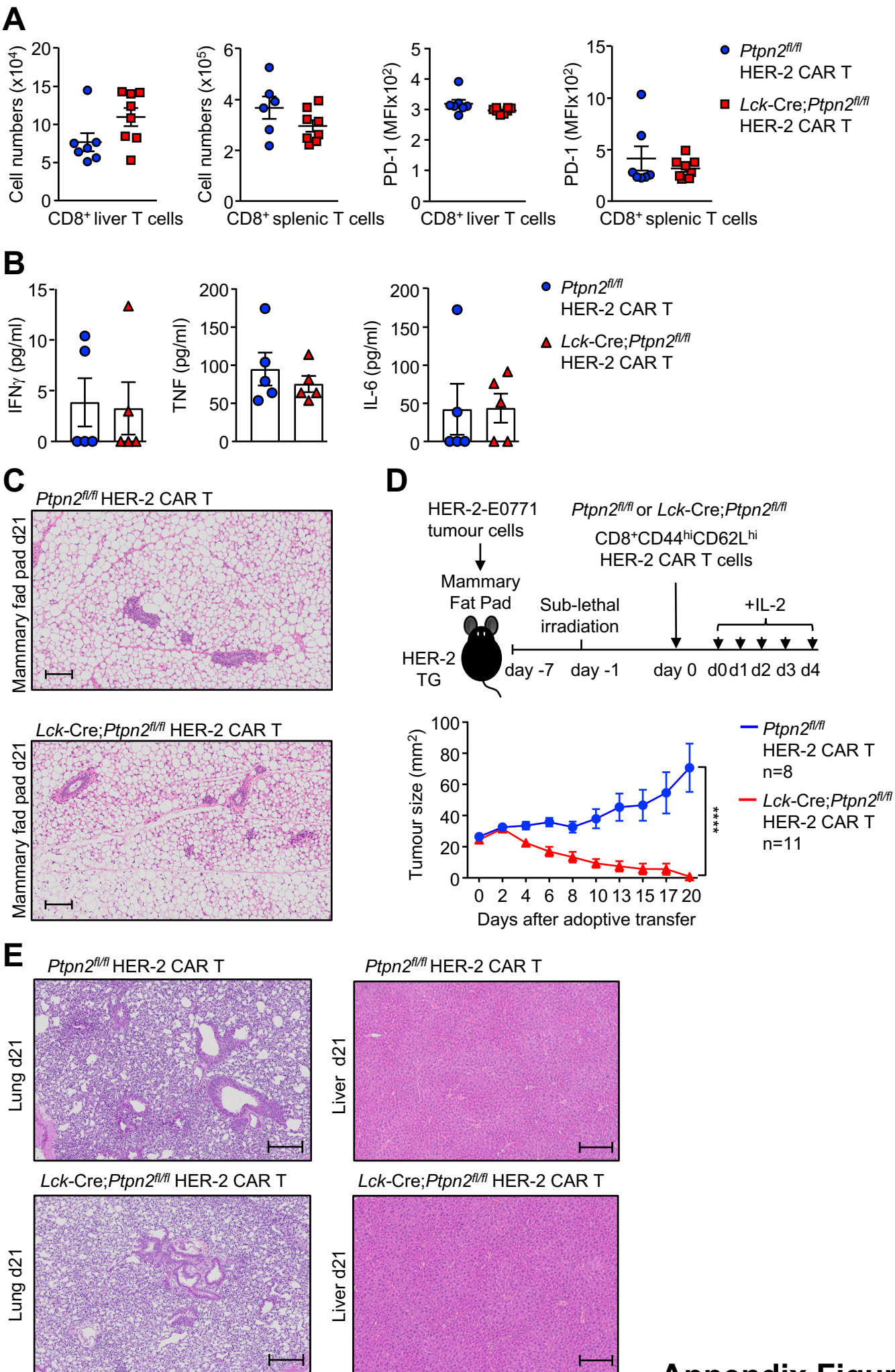
Appendix Figure S2



Appendix Figure S3

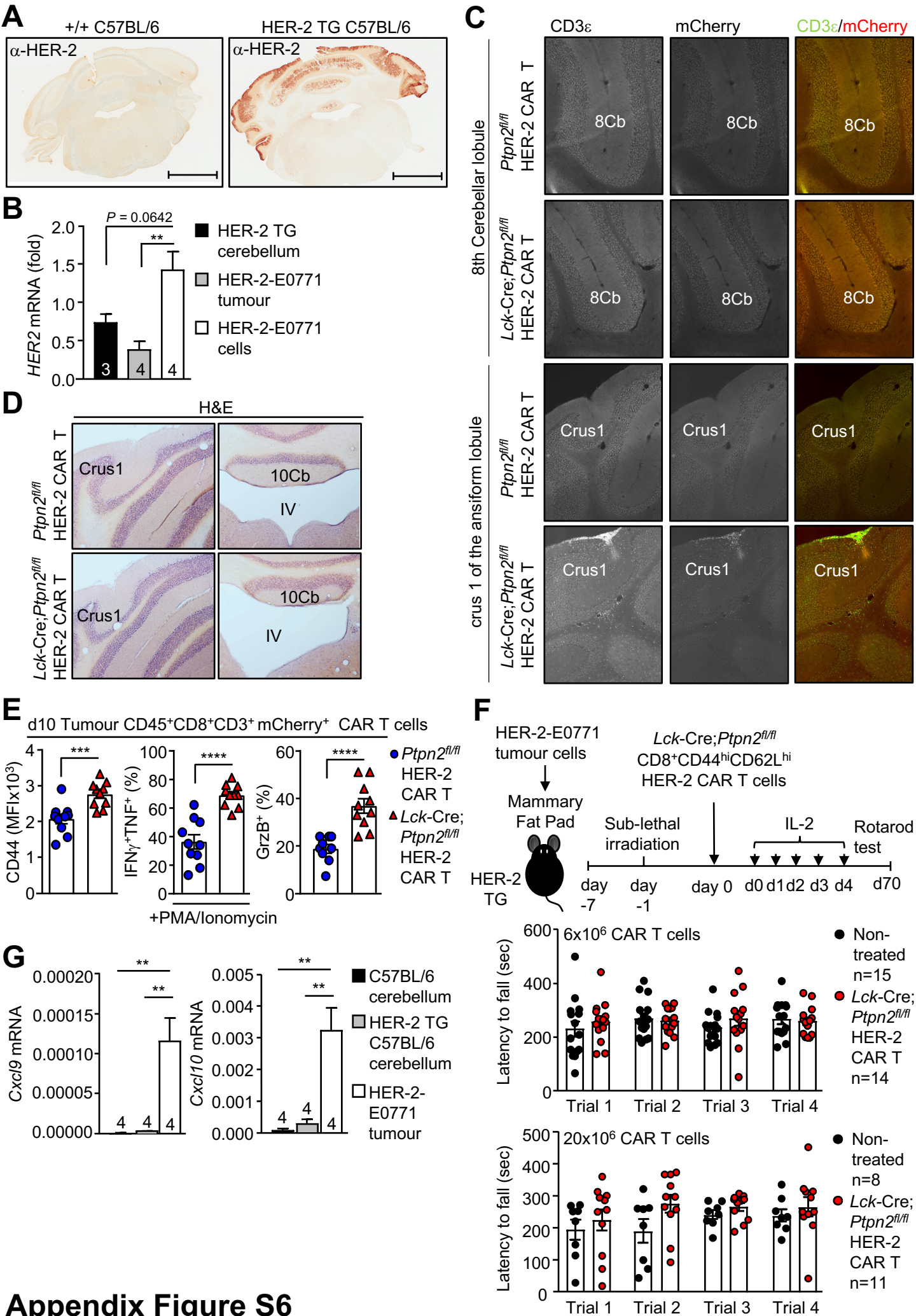


Appendix Figure S4



Appendix Figure S5





Appendix Figure S6



**Table EV1. Pathology in *Ptpn2<sup>fl/fl</sup>;p53<sup>+/-</sup>* mice**

| Mouse ID | Hepatoma | Sarcoma/<br>Carcinoma | Thymoma | Lymphoma | Splenomegaly | B1 cell<br>leukemia | Thymic DP T<br>cell leukemia | Splenic/Hepatic<br>DP T cell leukemia |
|----------|----------|-----------------------|---------|----------|--------------|---------------------|------------------------------|---------------------------------------|
| #1       | No       | No                    | Yes     | No       | No           | No                  | Yes                          | Yes                                   |
| #2       | No       | No                    | No      | Yes      | Yes          | Yes                 | No                           | No                                    |
| #4       | No       | No                    | No      | No       | No           | Yes                 | No                           | No                                    |
| #6       | No       | No                    | No      | No       | Yes          | Yes                 | No                           | No                                    |
| #7       | No       | Yes                   | No      | No       | No           | No                  | Yes                          | Yes                                   |
| #8       | No       | No                    | No      | No       | No           | No                  | Yes                          | No                                    |
| #14      | Yes      | No                    | No      | No       | Yes          | No                  | No                           | No                                    |
| #15      | No       | No                    | No      | No       | Yes          | Yes                 | No                           | No                                    |
| #18      | No       | Yes                   | No      | No       | No           | No                  | No                           | No                                    |
| #20      | No       | No                    | Yes     | No       | No           | No                  | Yes                          | Yes                                   |
| #23      | No       | No                    | Yes     | No       | No           | No                  | Yes                          | Yes                                   |
| #27      | No       | No                    | No      | No       | Yes          | Yes                 | No                           | No                                    |
| #30      | No       | Yes                   | No      | No       | No           | No                  | No                           | No                                    |
| #32      | No       | No                    | No      | No       | Yes          | Yes                 | No                           | No                                    |
| #34      | Yes      | No                    | No      | No       | No           | No                  | No                           | No                                    |