Experimental investigation of hematological toxicity after radiation therapy combined with immune checkpoint inhibitors

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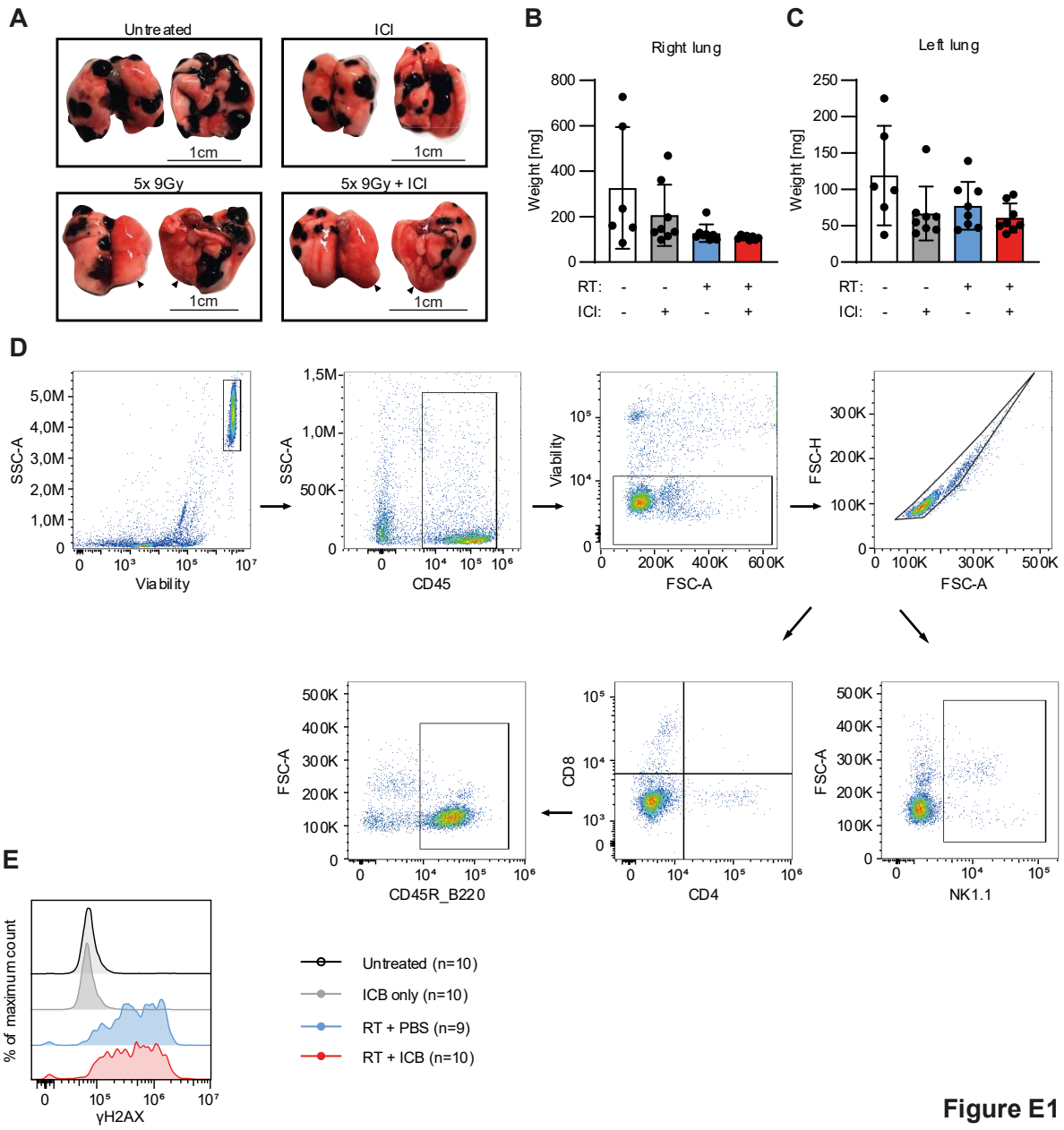

Figure E1

Supplementary Figure 1: Tumor burden after combined radioimmunotherapy of the lungs and details of flow cytometry blood cell analysis

(A-E) C57BL/6 mice received B16.OVA melanoma cells intravenously 4 days before the start of therapy: Fractionated RT (5 x 9 Gy) to the right thorax ± anti-PD-1 and anti-CTLA-4 (4 injections, weekly, starting in parallel with RT). **(A)** Representative images of lungs (front and back) isolated on day 22 after onset of RT, and **(B-C)** the weight of the lungs. The arrow

indicates the right lung that received RT. **(D)** Gating strategy for blood cell analysis by flow cytometry. After identifying beads, $CD45^+$ leukocytes, live and single, the identification and analysis of the relative abundances of $CD4^+$, $CD8^+$, $B220^+$, and $NK1.1^+$ cells are performed. Counting beads were used to assess the amount of the analyzed sample in relation to the total sample volume to calculate the absolute concentrations of respective immune cells per microliter of blood. (**E**) Representative histograms presenting the intensity of phosphorylated γH2AX signal of respective immune cell subpopulations assessed by flow cytometry directly after completing last fraction of RT. The mean fluorescence intensity (MFI) is presented. The figures shows data from one experiment analyzed using one-way ANOVA. Data are presented as mean ± SD. The number of mice (n) is shown in the figure. P values are presented in the figure.

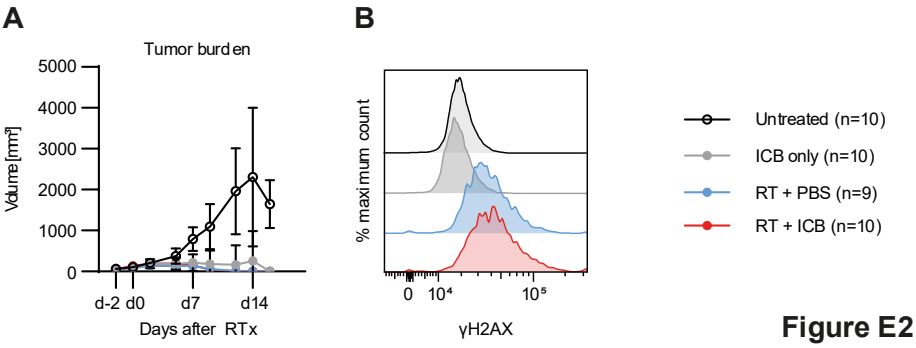

Figure E2

Supplementary Figure 2: Tumor burden after combined radioimmunotherapy of peripheral tumors in the upper hind legs and details of flow cytometry blood cell analysis.

(A-B) BALB/c mice received subcutaneous CT26 tumor cell injections into both upper hind legs one week before therapy: Fractionated RT (3 x 15 Gy) to both upper hind legs ± anti-PD-1 and anti-CTLA-4 (4 injections, twice per week, starting one day prior to RT). **(A)** Total tumor burden of mice after tumor cell injection (day -7) and following therapy starting on day -1. (**B**) Representative histograms presenting the intensity of phosphorylated γH2AX signal of respective immune cell subpopulations assessed by flow cytometry directly after completing last fraction of RT. The mean fluorescence intensity (MFI) is presented. Pooled data from 2 independent experiments. The data were analyzed using one-way ANOVA and are presented as mean ± SD. The number of mice (n) is shown in the figure. P values are presented in the figure.