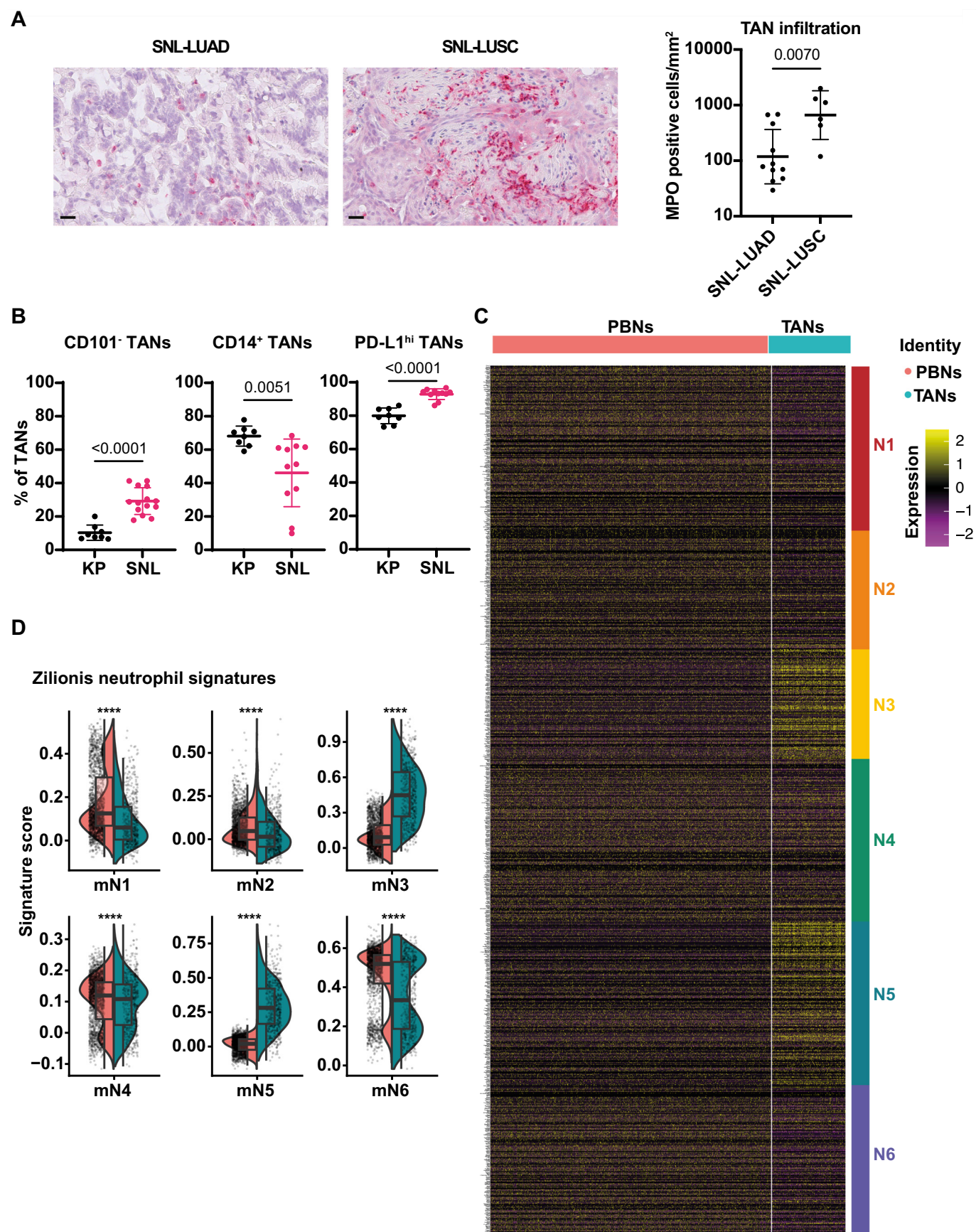


Expanded View Figures

Figure EV1. Variability between TANs in LUSC compared to LUAD.

(A) (Left) Representative MPO staining and (Right) quantification in LUAD and LUSC lesions of SNL mice ($n = 11$ for LUAD and $n = 6$ for LUSC). Scale bars: 20 μm . (B) Comparison of frequency of CD101⁺ (left), CD14⁺ (middle) and PD-L1^{hi} (right) TANs in KP ($n = 8$ tumors) and SNL ($n = 11$ –14 tumors). (C) Heatmap depicting expression of individual genes by single-cell RNA-seq (scRNA-seq) of PBNs ($n = 1702$ cells) and TANs ($n = 445$ cells) from SNL mice. Genes are categorized by neutrophil subsets “N1”, “N2”, “N3”, “N4”, “N5”, and “N6”, as defined previously from murine scRNA-seq data (Data ref: Zilionis et al, 2019). (D) Split violin plots depicting expression of indicated neutrophil subset signature in scRNA-seq data of PBNs compared to TANs of SNL mice. Data information: p -value was determined using unpaired t-test for (A) and (B left and right), Welch’s t-test for (B middle). For (D), Wilcoxon rank-sum test was used (**** $p < 3.3\text{E-}0.5$). Data are shown as mean $\pm$ SD (A, B). For (D), box and whisker plot overlays depict median and upper and lower quartiles of expression. Each dot represents an individual cell.



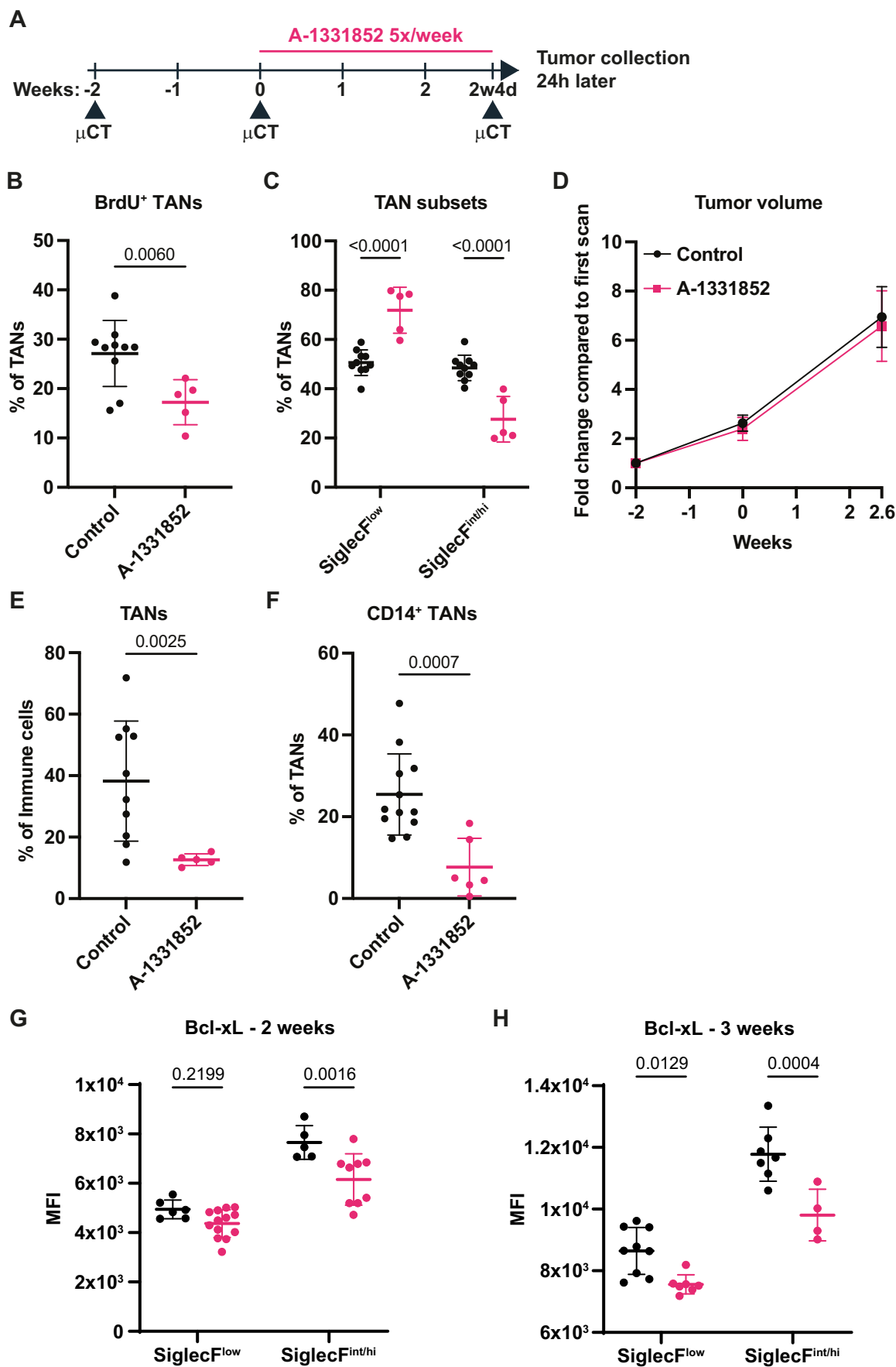
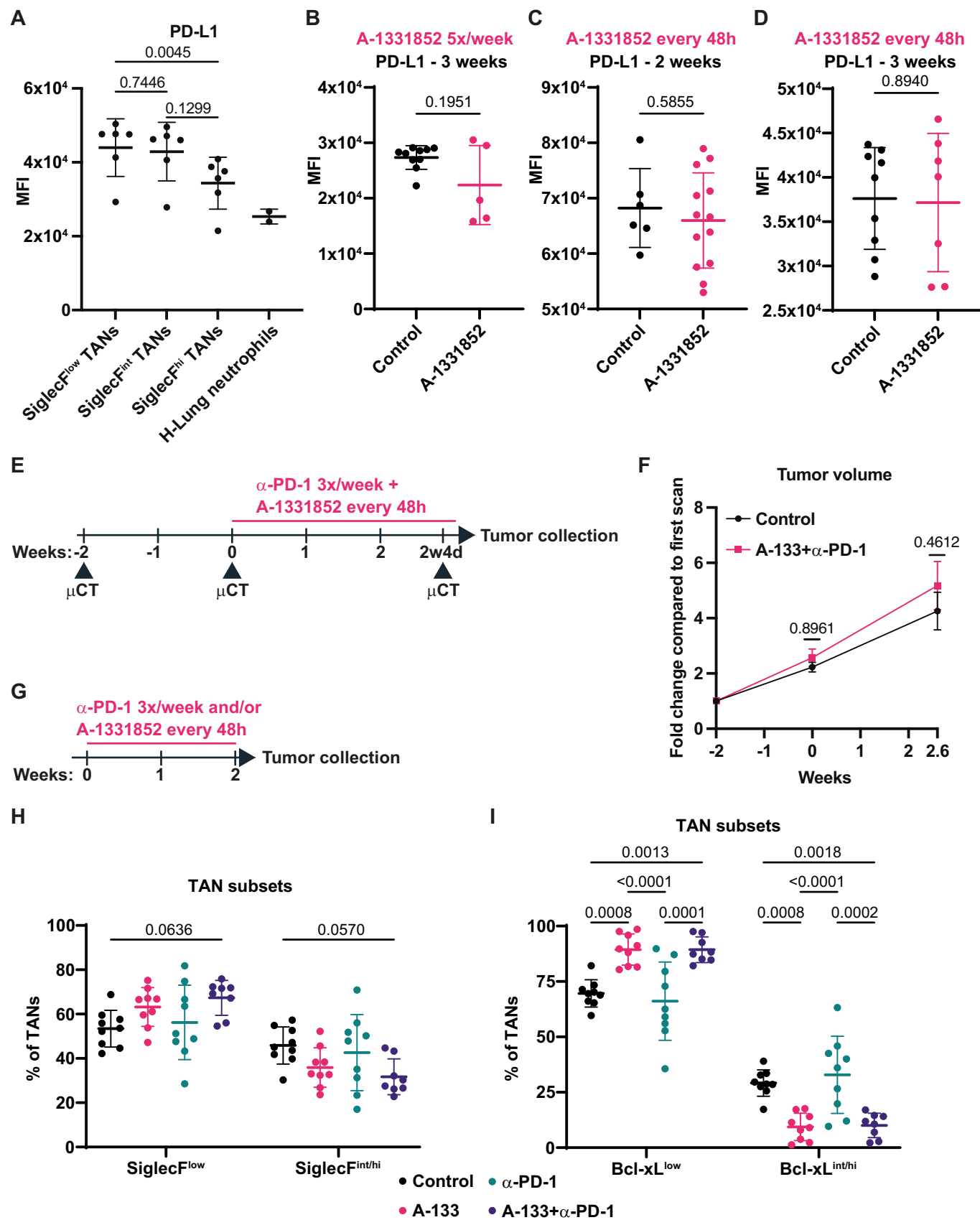


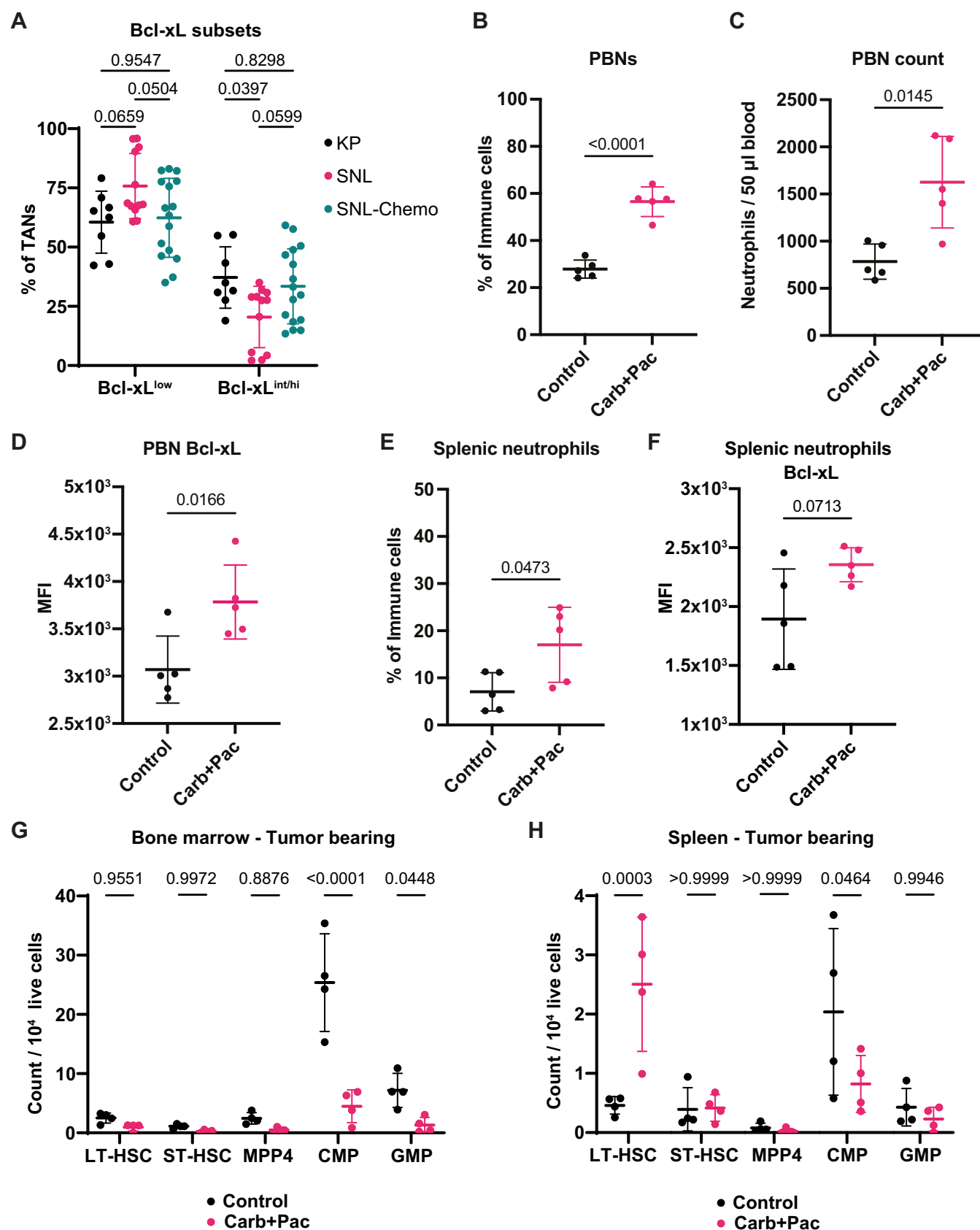

Figure EV2. Bcl-xL blockade 5x/week does not diminish SNL tumor growth.

(A) Scheme of in vivo Bcl-xL blockade regimen. (B) Proportions of 6.5-day-old BrdU⁺ TANs among total TANs ($n = 10$ for control and $n = 5$ for treated group). (C) Proportions of SiglecF TAN subsets among total TANs ($n = 10$ tumors for control and $n = 5$ for treated group). (D) Tumor growth kinetics measured by μ CT ($n = 11$ tumors for control group and $n = 6$ tumors for treated group). (E) Proportions of TANs among all immune cells ($n = 10$ tumors for control and $n = 5$ for treated group). (F) Proportions of CD14 TANs among total TANs ($n = 12$ tumors for control and $n = 6$ tumors for treated). (G) MFI of Bcl-xL expression in SiglecF^{low} ($n = 6$ tumors for control and $n = 13$ for treated group) and SiglecF^{int/hi} ($n = 5$ for control and $n = 9$ for treated group) TANs. (H) MFI of Bcl-xL expression in SiglecF^{low} ($n = 9$ tumors for control and $n = 7$ for treated group) and SiglecF^{int/hi} ($n = 7$ for control and $n = 4$ for treated group) TANs. Data information: p -value was determined using Welch's t -test for (B), (E), and (F). For (C), (D), (G), and (H), 2-way ANOVA was used. Data are shown as mean $\pm$ SD except for (D), which is shown as mean $\pm$ SEM.



◀ **Figure EV3. Combined Bcl-xL blockade and anti-PD-1 does not diminish tumor growth.**

(A) MFI of PD-L1 expression among TAN subsets ($n = 6$ for matched tumors and $n = 2$ for healthy lung (H-Lung) neutrophils). (B) MFI of PD-L1 expression in TANs upon 5x/week A-1331852 regimen for 3 weeks ($n = 10$ tumors for control and $n = 5$ for treated group). (C) MFI of PD-L1 expression in TANs upon 1x/48 h A-1331852 regimen for 2 weeks ($n = 6$ tumors for control and $n = 13$ for treated group). (D) MFI of PD-L1 expression in TANs upon 1x/48 h A-1331852 regimen for 3 weeks ($n = 9$ tumors for control and $n = 7$ for treated group). (E) Scheme of anti-PD-1 immunotherapy combined with A-1331852. (F) Tumor growth kinetics measured by μ CT ($n = 12$ tumors for control group and $n = 8$ tumors for treated group). (G) Scheme of anti-PD-1, A-1331852 or their combination for 2 weeks. (H) Proportion of SiglecF TAN subsets among total TANs ($n = 8-9$ tumors per group). (I) Proportion of Bcl-xL TAN subsets among total TANs ($n = 8-9$ tumors per group). Data information: p -value was determined using Friedman test for (A). For (B), Welch t-test was used and for (C), and (D), unpaired t-test was used. For (F), (H), and (I), 2-way ANOVA was used. Data are shown as mean $\pm$ SD except for (F), which is shown as mean $\pm$ SEM.



◀ Figure EV4. Combination chemotherapy increases circulating neutrophil numbers and their expression of Bcl-xL.

(A) Proportion of Bcl-xL TAN subsets among total TANs ($n = 8$ –16 tumors per group). (B) Proportions of PBNs among immune cells in control group or carboplatin and paclitaxel (Carb+Pac) treated group. (C) Absolute PBN counts per 50 μ l of blood. (D) MFI of Bcl-xL expression in PBNs. (E) Splenic neutrophil proportions among immune cells. (F) MFI of Bcl-xL expression in splenic neutrophils. (G, H) Quantities of hematopoietic precursors in the bone marrow (G) and spleen (H) among 10^4 live cells ($n = 4$ mice per group). Data information: p -value was determined using 2-way ANOVA for (A), (G), and (H). For (B), (C), (D), (E), and (F), Welch's t -test was used ($n = 5$ per group). Data are shown as mean $\pm$ SD.

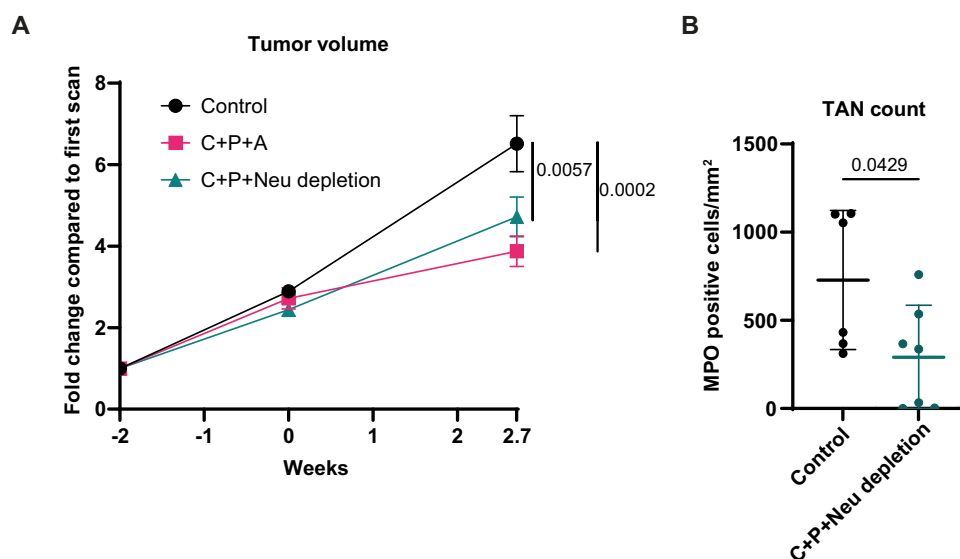


Figure EV5. Partial neutrophil depletion is comparable to A-1331852 in the anti-tumor response to chemotherapy.

(A) Tumor growth kinetics measured by μ CT ($n = 9-14$ tumors per group). (B) IHC Quantification of MPO positive cells per mm² of tumor ($n = 6$ tumors for control and $n = 7$ for neutrophil depleted group). Data information: p -value was determined using 2-way ANOVA for (A) and unpaired t -test for (B). Data is shown as $\pm$ SEM for (A) and $\pm$ SD for (B).