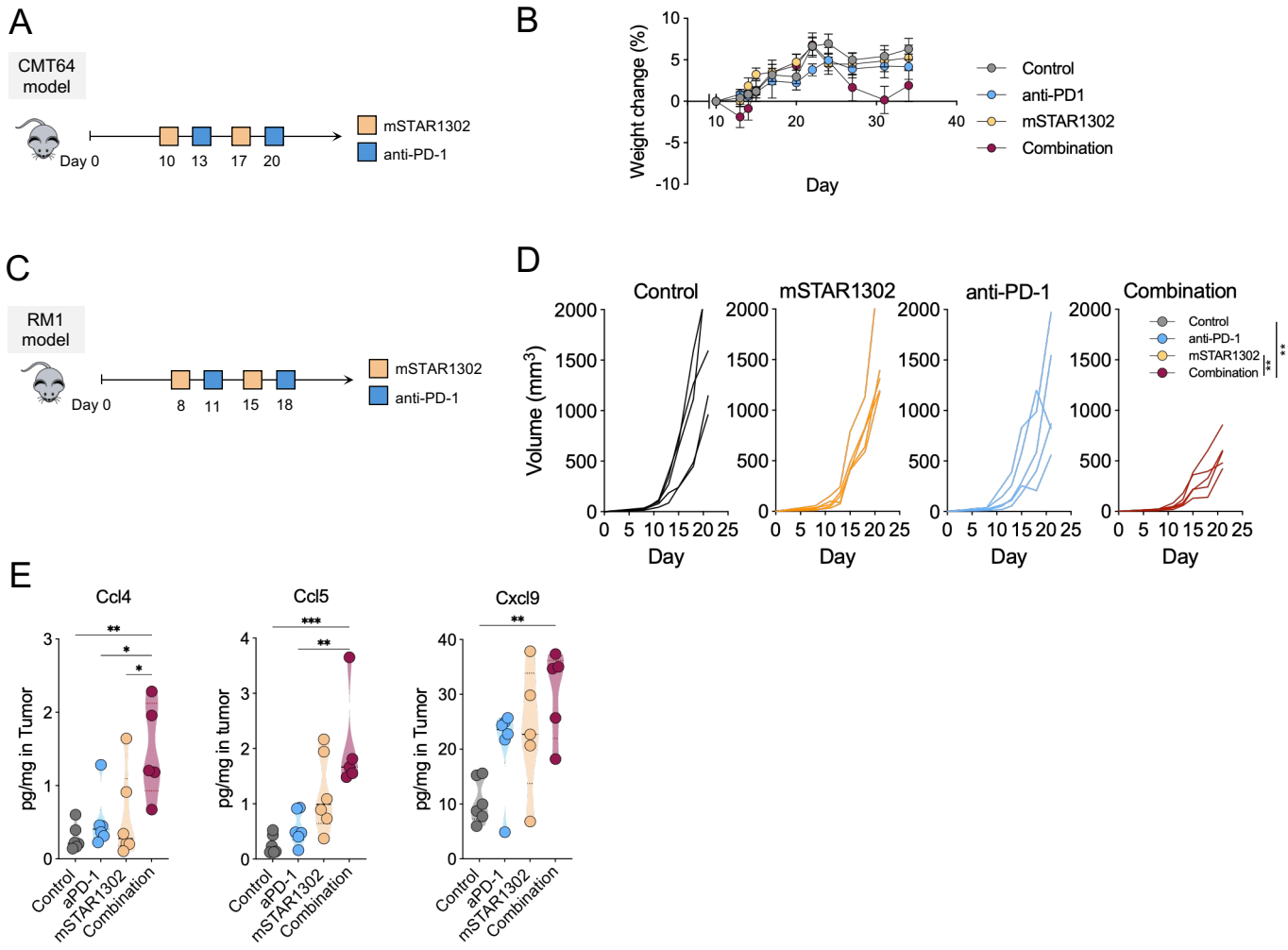
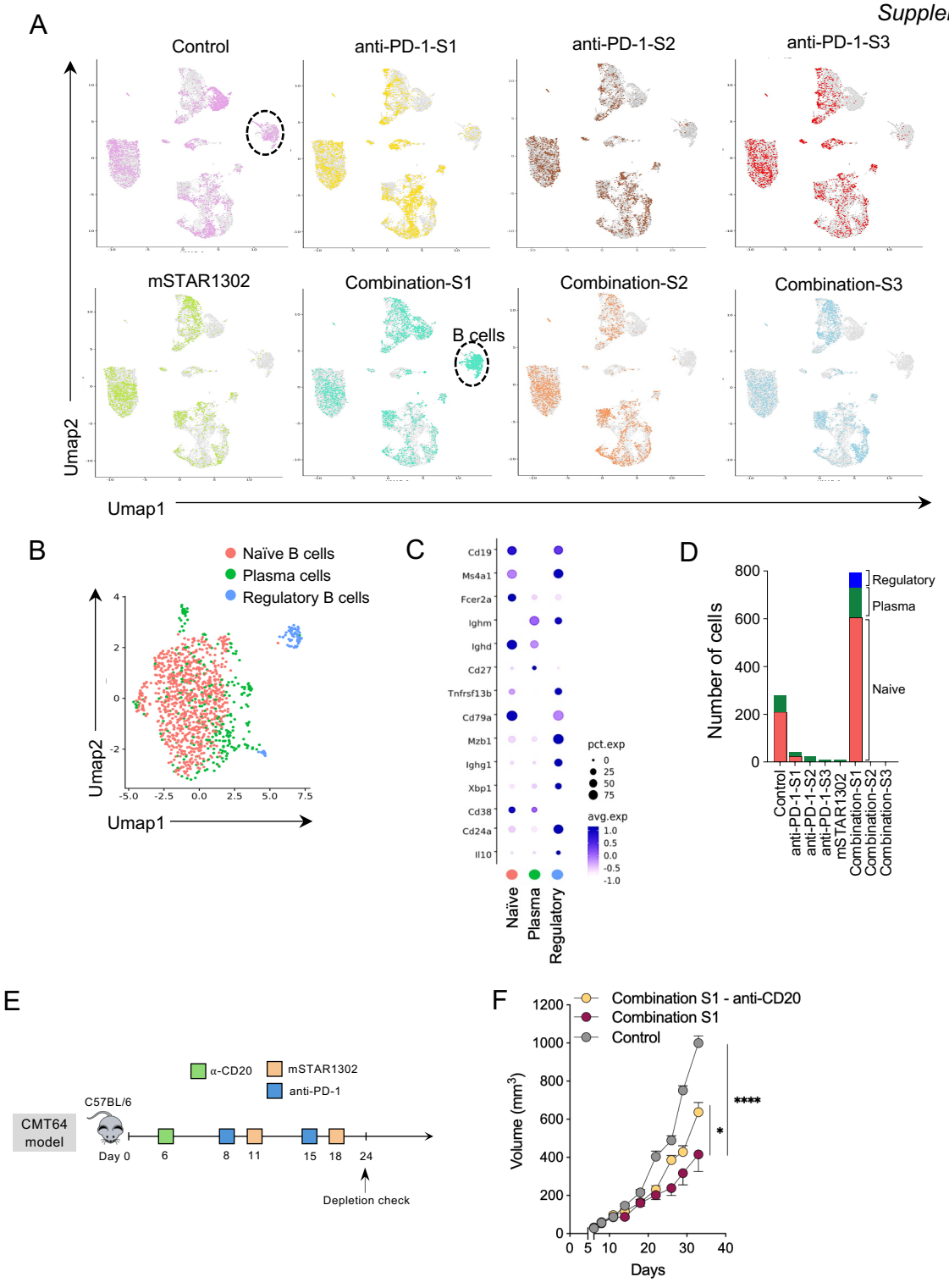
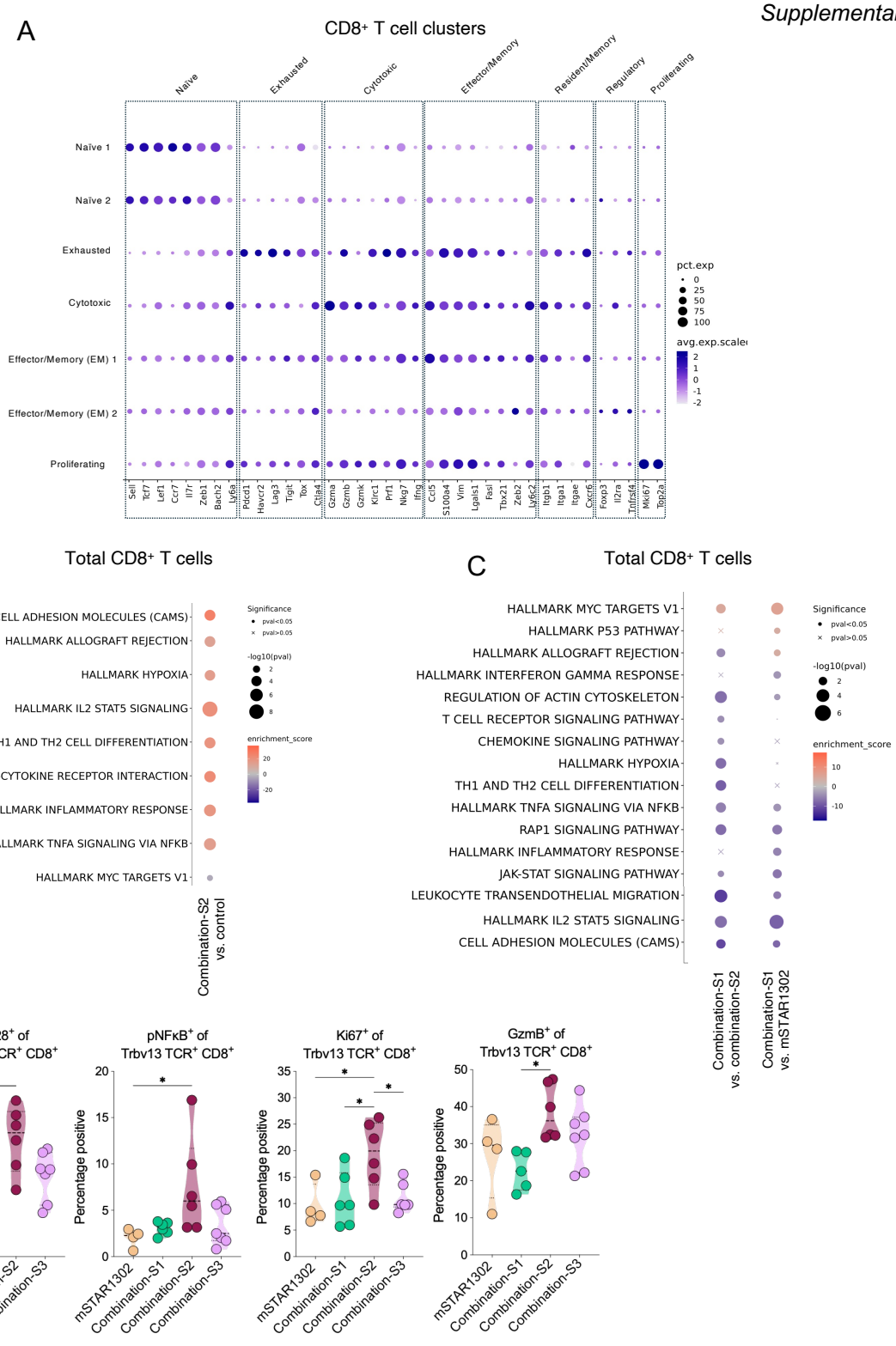


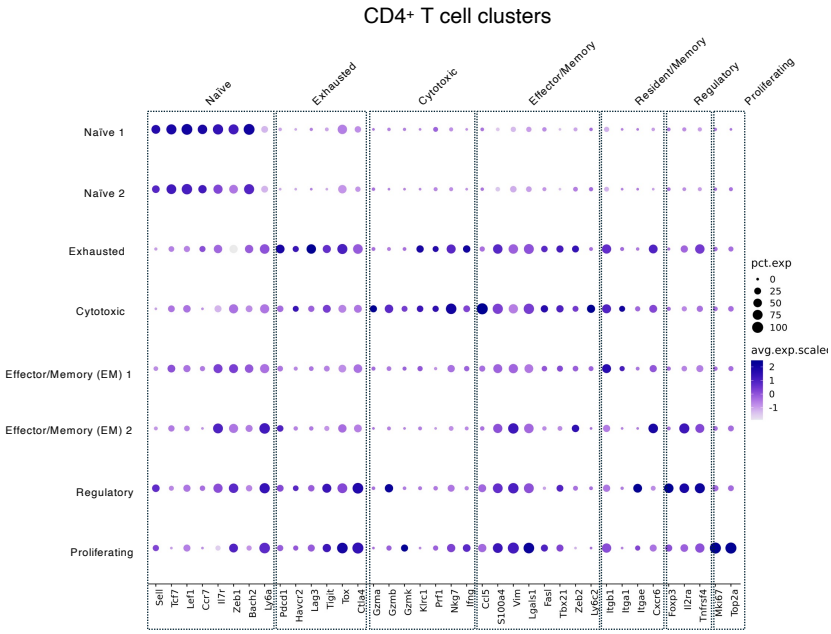
Supplemental Figure 1. Safety, anti-tumor effect, and mechanisms of the combination mSTAR1302 and PD-1 blockade. (A) Schema of treatment of mice bearing CMT64 tumors. Treatment began when the tumors reached 70 mm^3 (n=7-8 mice per group). **(B**) Average percent weight change for mice in each treatment group. (**C**) RM-1 prostate tumor cells were implanted subcutaneously (s.c.) in C57BL/6 mice; following randomization, treatments started when tumors reached ~30 mm^3. Mice were given two cycles of 20 µg mSTAR1302 and 200 µg PD-1 blockade antibody following the combination schedule 2 (treatment began with mSTAR1302). (**D**) Individual tumor growth curves; n=5 mice per group. Statistical analysis was conducted as indicated in Materials and Methods. (**E**) Analysis of cytokines and chemokines in CMT64 tumors treated as in Fig. 3A via the Olink assay. Data are presented with the medians and quartiles indicated by dashed lines, statistical significance was calculated by one-way ANOVA with Tukey's post hoc test.



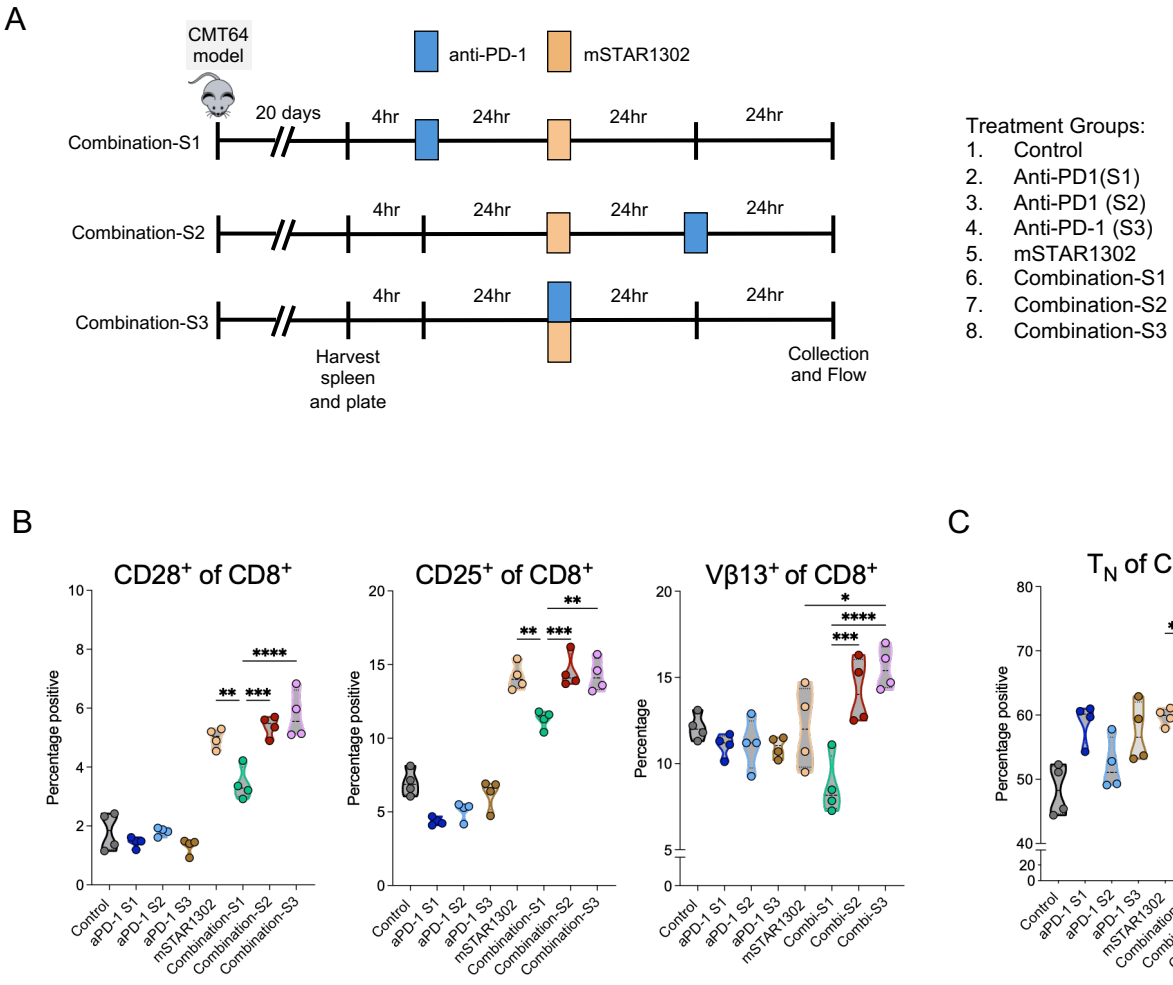
Supplemental Figure 2. Transcriptomic analysis revealed a regulatory B-cell population within tumors treated with PD-1 blockade prior to mSTAR1302 administration. (**A**) UMAPs highlighting cells according to treatment group. A population of B cells was observed in the control and combination-S1 groups. (**B**) UMAP of 1,177 B cells re-clustered after subsetting. Phenotype annotation for each cluster was identified by gene expression. (**C**) Gene expression bubble plot used to phenotype B cell clusters in B. (**D**) Stacked bar graph presenting B-cell numbers and phenotype across the untreated and treated groups. (**E**) CMT64 cells were implanted s.c.; mice were administered 250 µg anti-CD20 on day 6. Animals were administered two cycles of 200 µg anti-PD-1 followed by 20 µg mSTAR1302 starting on day 8. (**F**) Anti-tumor activity of the combination-S1 with and without B-cell depletion. Data presented are average tumor volumes ± SEM, n=6-8 mice per group, significance was calculated by two-way ANOVA with a mixed-effects model considering both time and tumor volume.



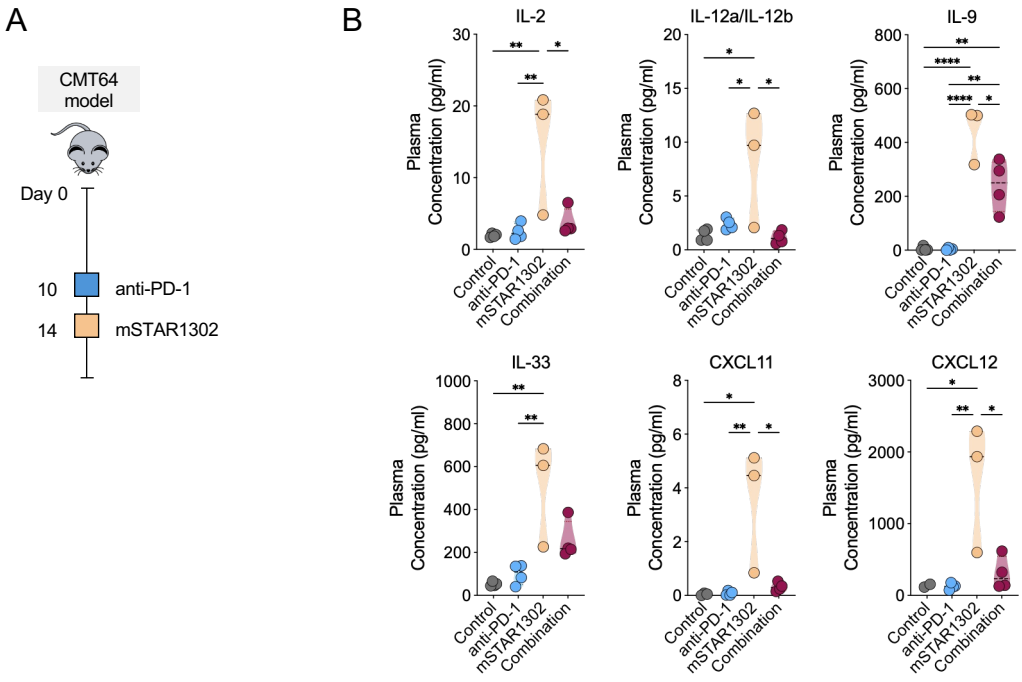
Supplemental Figure 3. Relative gene expression comparisons to identify T cell phenotypes. (**A**) Gene expression bubble plots used to identify cluster phenotypes for CD8+ T cells. (**B, C**) Significant pathways differentially modulated on CD8+ T cells in the combination-S2 vs. control (B), or the combination-S1 vs. -S2 or mSTAR1302. (**D**) Flow cytometry-based immunophenotyping of TIL collected from CMT64 tumors. Only the mSTAR1302 monotherapy and the 3 combination therapy groups are shown; n=4-7 tumors harvested from each treatment group, data presented with the median and quartiles marked by dashed lines, significance calculated by one-way ANOVA with Tukey's post hoc test.



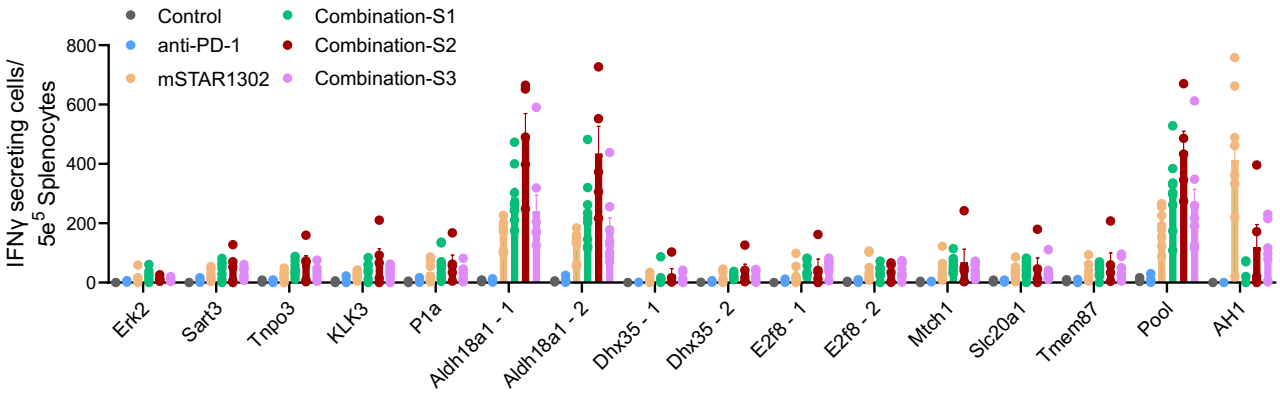
Supplemental Figure 4. Relative gene expression comparisons to identify T-cell phenotypes. Gene expression bubble plots used to identify cluster phenotypes for CD4+ T cells.



Supplemental Figure 5. In vitro activation of splenocytes from CMT64 tumor-bearing mice activated with various schemas of combination therapy. (**A**)Splenocytes were harvested from a CMT64 tumor-bearing mouse on day 20 after implantation and activated with indicated agents following the procedure described in the Materials and Methods. mSTAR1302 was administered at the same time point across the three schedules, using final concentrations of 29.3 μg/ml anti-PD-1 (BioXcell) and 2.93 μg/ml mSTAR1302. (**B, C**) Cells were collected and stained for flow cytometry analysis for indicated markers. Significance was calculated by one-way ANOVA with Tukey's post-hoc test.



Supplemental Figure 6. PD-1 blockade administered prior to mSTAR1302 precludes release of systemic cytokines and chemokines. (**A**) CMT64 lung tumor cells were implanted subcutaneously, and mice were randomized and treated when tumors reached ~70mm^3. Mice were treated with one cycle of 200 μg anti-PD-1 antibody and 20 μg mSTAR1302 following the Schedule 1 in which treatment began with PD-1 blockade. Blood was harvested via submandibular bleeds on day 16 to isolate plasma, n=3-5 mice per group. (**B**) Plasma concentration of cytokines and chemokines determined by Olink proteomics. Significance was calculated by one-way ANOVA with Tukey's post-hoc test.



Supplemental Figure 7. ELISpot assay results for each individual peptide in the neoantigen-containing pool shown in Fig. 7g. Antigen-specific T cells quantified by ELISpot are reported as IFNγ-secreting cells/5x10^5 splenocytes; n=5-9 mice per treatment group, data presented as mean ± SEM indicated by bars and individual data points overlayed.