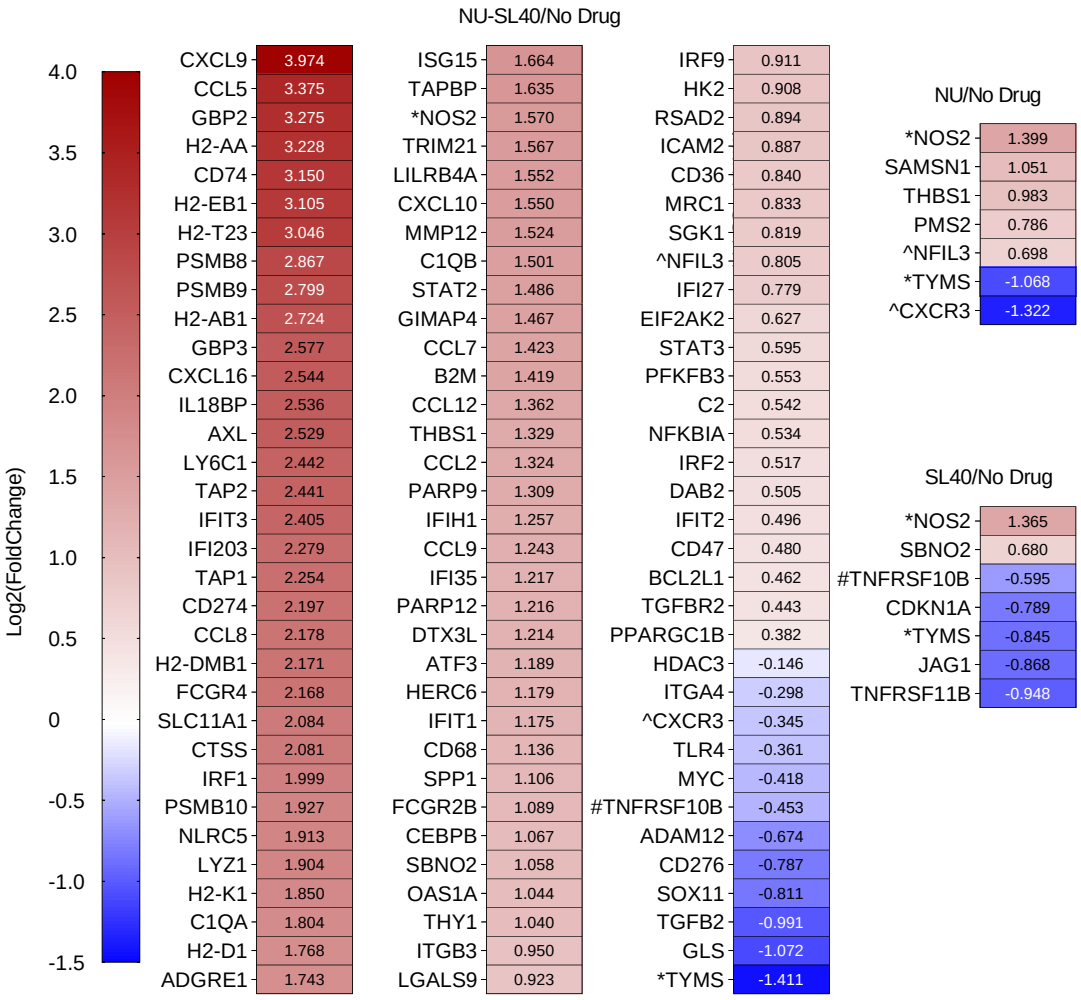
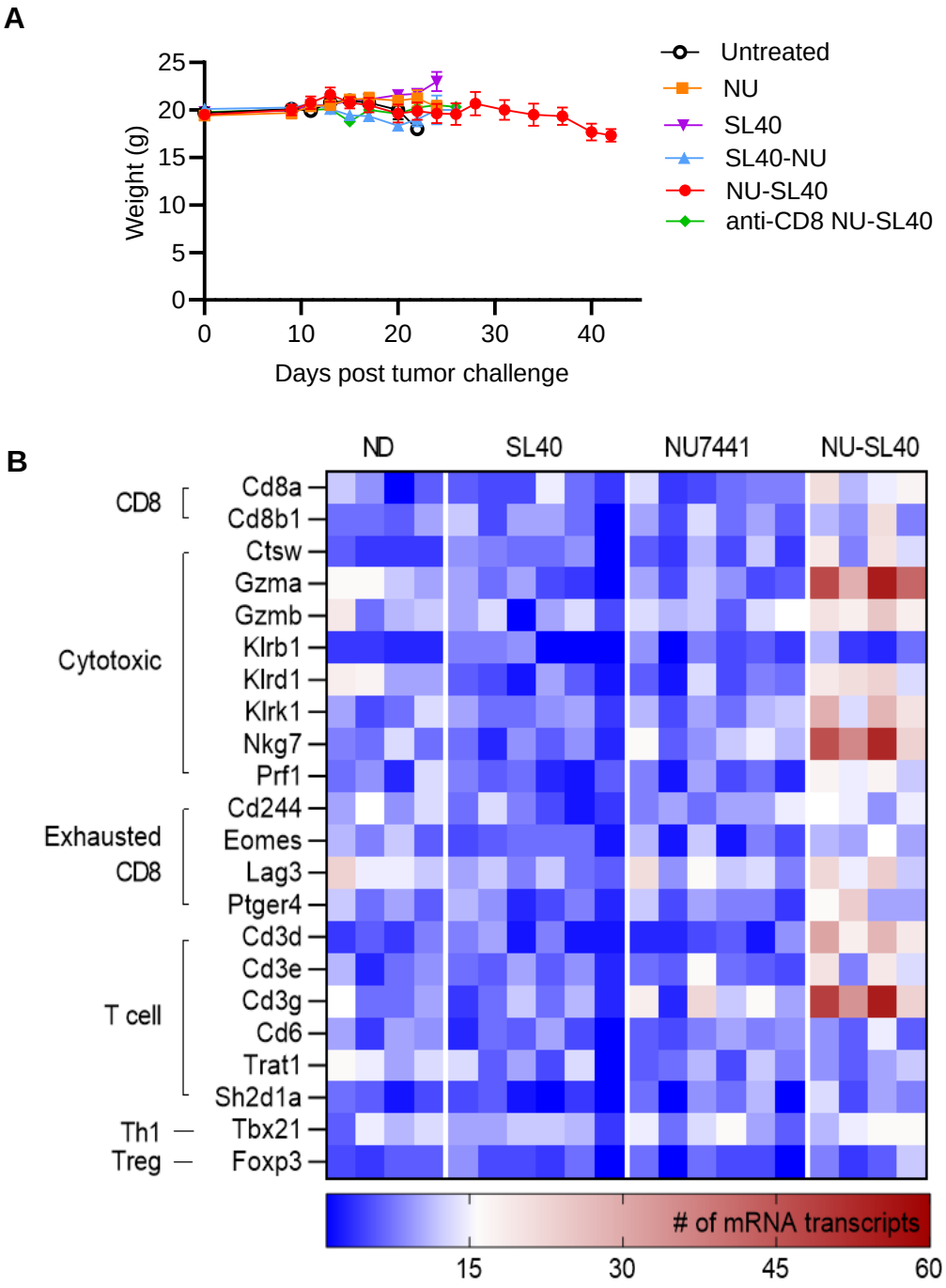
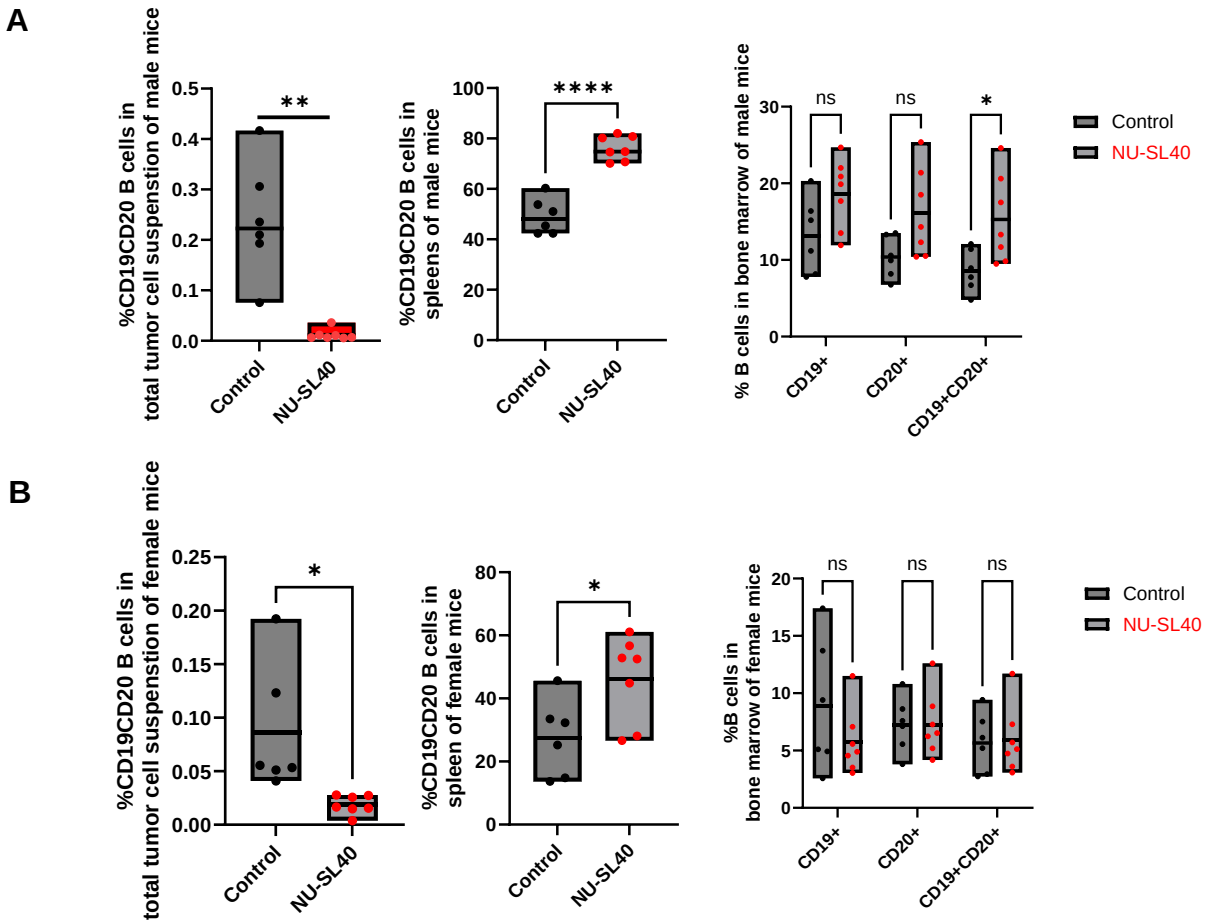


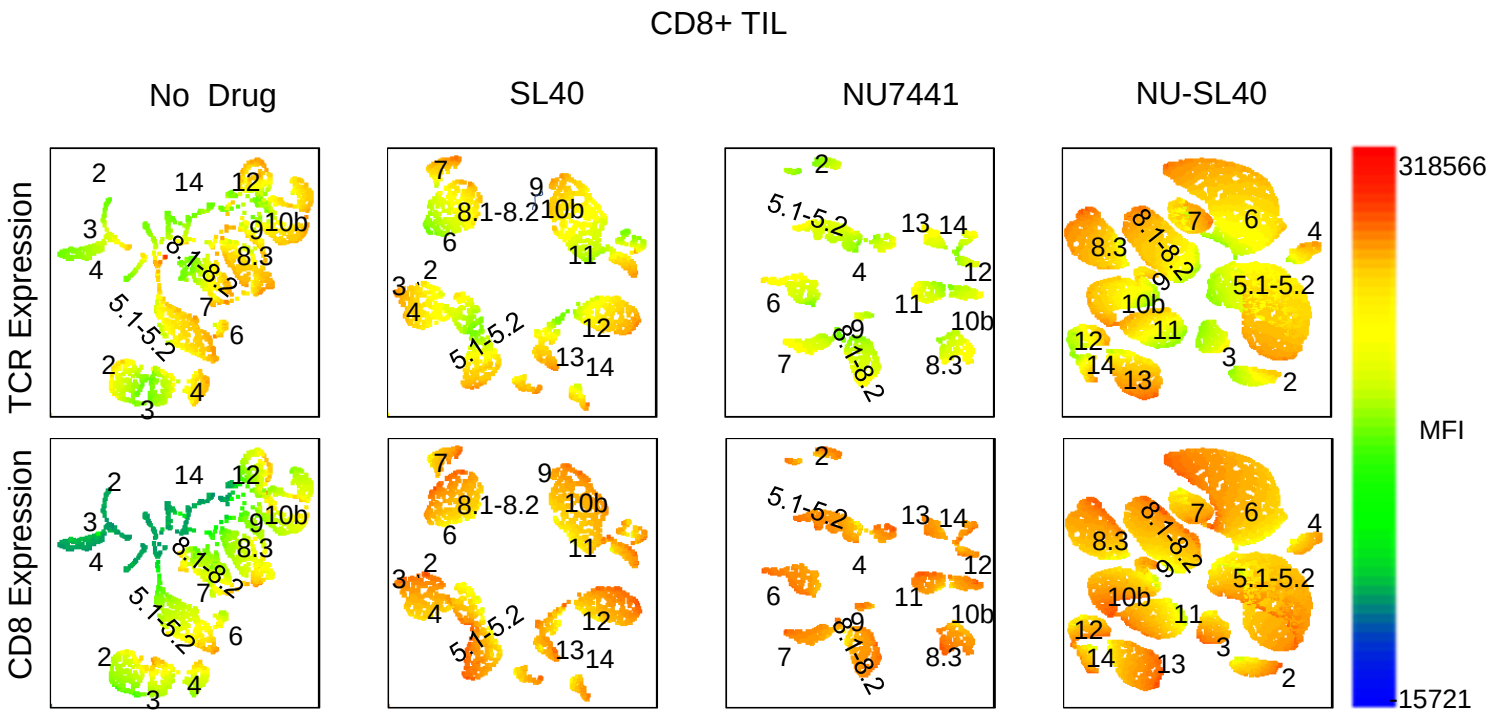
Supplemental Table 1 – Nanostring Pan Cancer IO differentially regulated gene expression. List of differentially regulated genes determined by Nanostring Pan Cancer IO Gene Expression Analysis. Only genes that were statistically significant (p-value < 0.05, fold-change more than +/- 1.5) were included. (*) Indicates gene differentially regulated in all treatments. (^) indicates gene differentially regulated in NU-SL40 and NU. (#) Indicates gene differentially regulated in NU-SL40 and SL40.



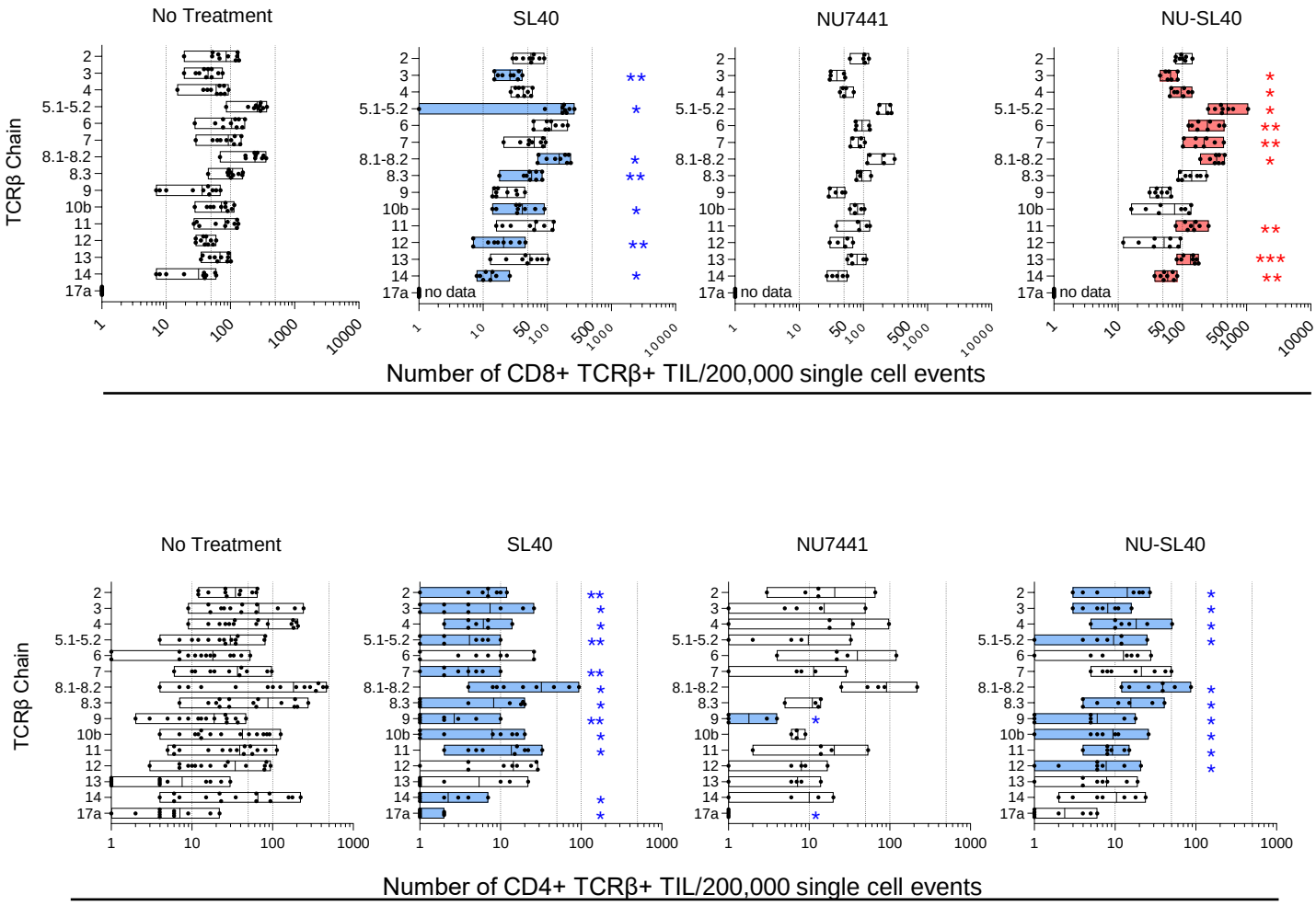
Supplemental Figure. 1 A) C57BL/6 mice were injected with B16-F10 tumor cells, and once tumors were established (25mm^2), mice were either left untreated or received NU alone, SL40 alone, combination SL40-NU, or combination NU-SL40. CD8 T cells were depleted by administration of anti-CD8 prior to treatment with NU-SL40. CD8 depletion was administered twice prior to NU-SL40 treatment. Weight represents group means +/- SEM – untreated, n=10; NU (n=10); SL40 (n=10); SL40-NU (n=11); NU-SL40 (n=8); CD8 depleted NU-SL40 (n=10). **B)** Heatmap of T cell associated genes by number of mRNA transcripts extracted from whole B16 tumors generated using Nanostring Pan Cancer IO gene expression panel.



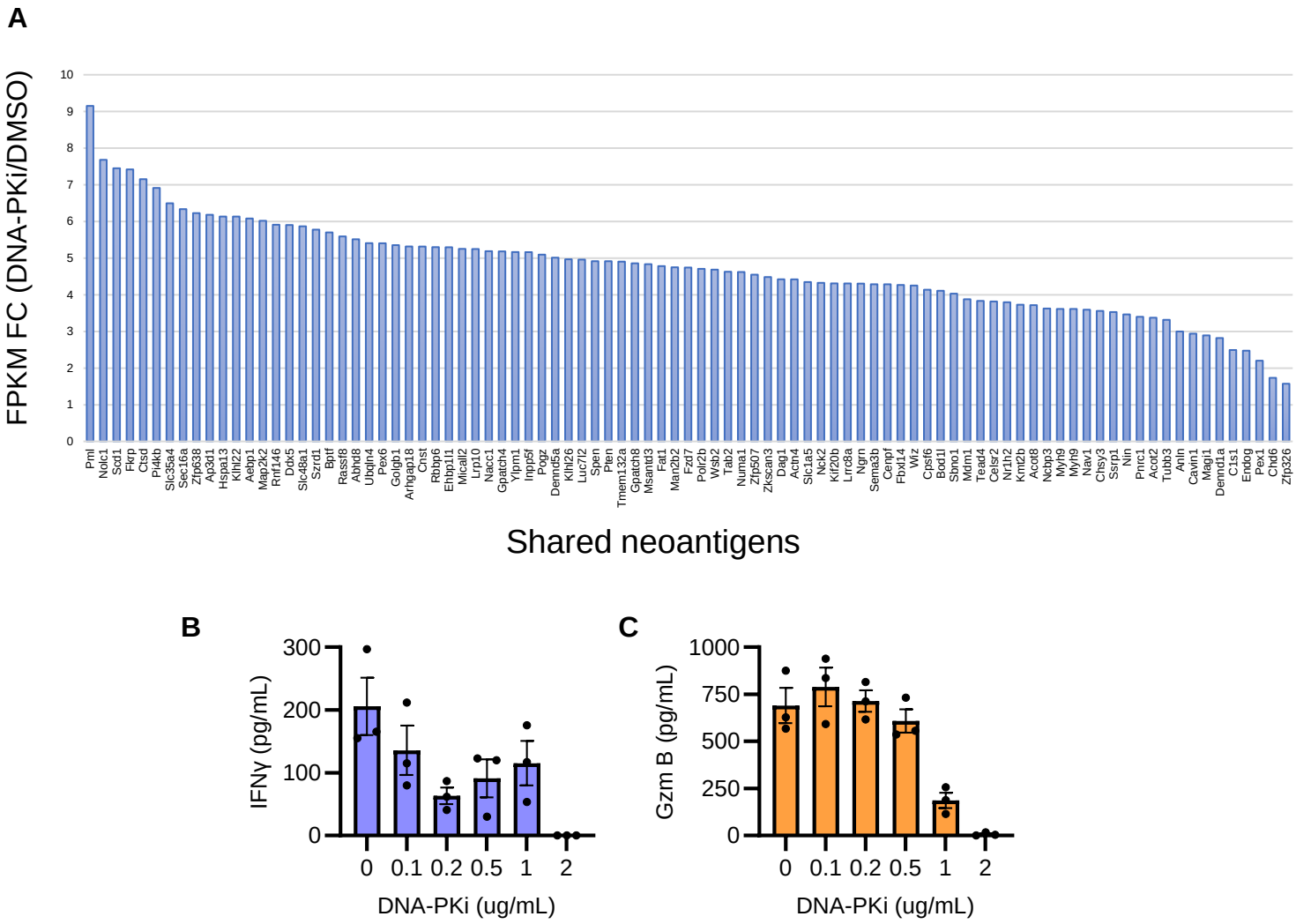
Supplemental Figure 2. B16-F10 tumor-bearing male or female mice were treated with or without combination NU-SL40 and 10 days after treatment, tissues were collected to analyze B cell populations by flow cytometry. Frequency of B cell populations in the tumor (left), spleen (middle), and bone marrow (right) in **A)** male and **B)** female mice. Untreated (n=6); NU-SL40 treated (n=7). Statistical tests were performed using student's *t* tests. **P* < 0.05, ***P* < 0.01, *****P* < 0.0001.



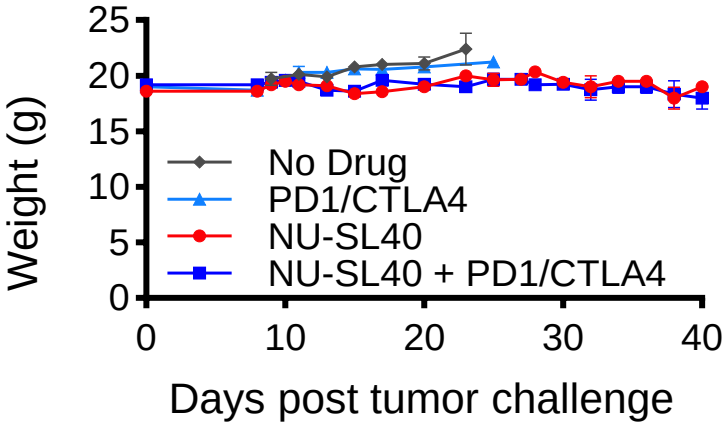
Supplemental Figure 3. C57BL/6 mice were injected with B16-F10 tumor cells, and once tumors were established (25mm^2), mice were either left untreated or received NU alone, SL40 alone, or combination NU-SL40. Ten days after mice received SL40, whole tumor samples were stained with antibodies against CD3, CD8, CD45, and the indicated TCRVβ. Plots show the raw distribution CD8 TCRVβ family members with relative TCR or CD8 protein density levels on the surface of specific CD45+CD3+CD8 TILs. Untreated (n=15); SL40 (n=9); NU (n=5); NU-SL40 (n=8).



Supplemental Figure 4. CD8 and CD4 TCRvβ repertoire distribution in the circulation of tumor-bearing mice. B16-F10 tumor-bearing mice were treated as described in Figure 1A and blood collected between 10 and 12 days post immune adjuvant vaccine. After red blood cell lysis, cells were stained with antibodies against CD3, CD4, and TCR and analyzed by flow cytometry. The number of TCRvβ chain per 200,000 single cell events is shown. Blue and red bars represent significant decreases or increases, respectively, in TCRvβ counts in treatment conditions compared to no treatment. Each dot represents data collected from one mouse. Untreated (n=5); SL40 (n=5); NU (n=5); NU-SL40 (n=4). Statistical significance determined by multiple unpaired t-tests. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

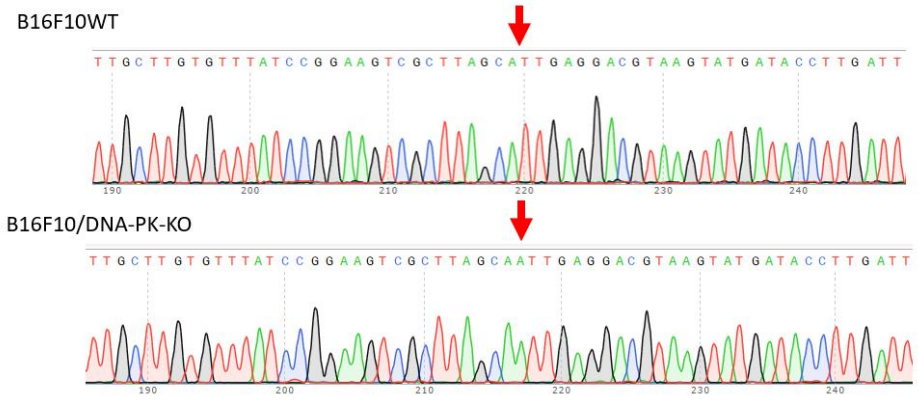


Supplemental Figure 5. A) B16-F10 melanoma cells were treated with DMSO and 2µM NU7441 for 72 hours and neoantigens identified as described previously (Lu et al. *Clin Cancer Res.* 2014;20(13):3401-10). The bar graph represents the FPKM levels of neoantigen-producing genes shared between DMSO and 2µM NU7441. Polyclonal TIL from human melanoma patient donor were activated with anti-CD3/-CD28 beads (1:20) with titrated concentrations of NU7441 (ug/mL) for 96hrs. Bar graphs indicate **B)** interferon gamma (1:800 dilution) and **C)** granzyme B (1:200 dilution) production by ELISA of triplicate samples (pg/mL).

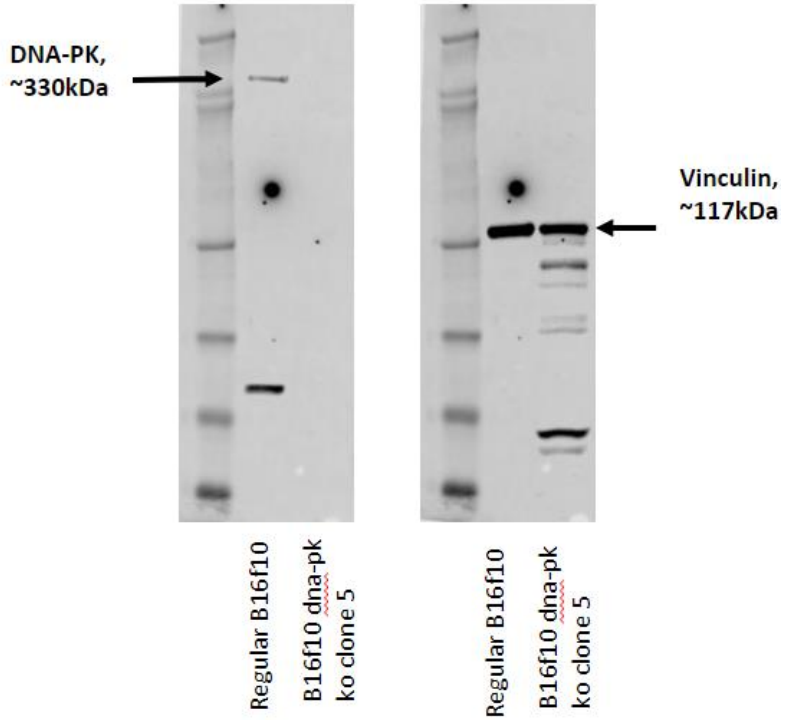


Supplemental Figure 6. C57BL/6 mice with established (~25mm^2) B16-F10 tumors remained untreated, treated with PD-1/CTLA-4 blockade, NU-SL40, or NU-SL40 in conjunction with PD-1/CTLA-4 blockade as described in the materials and methods section. Mouse weight (grams) was monitored over time.

A



B



Supplemental Figure 7. DNA-PK was knocked out in B16-F10 melanoma cells using CRISPER/Cas9 vectors targeting exon #3. **A)** Genomic sequencing data confirming mutation in the indicated nucleotide (red arrow). **B)** Western blot analyses to confirm the knocking out of DNA-PK in B16-F10 (left panel) and equal protein loading (right panel).