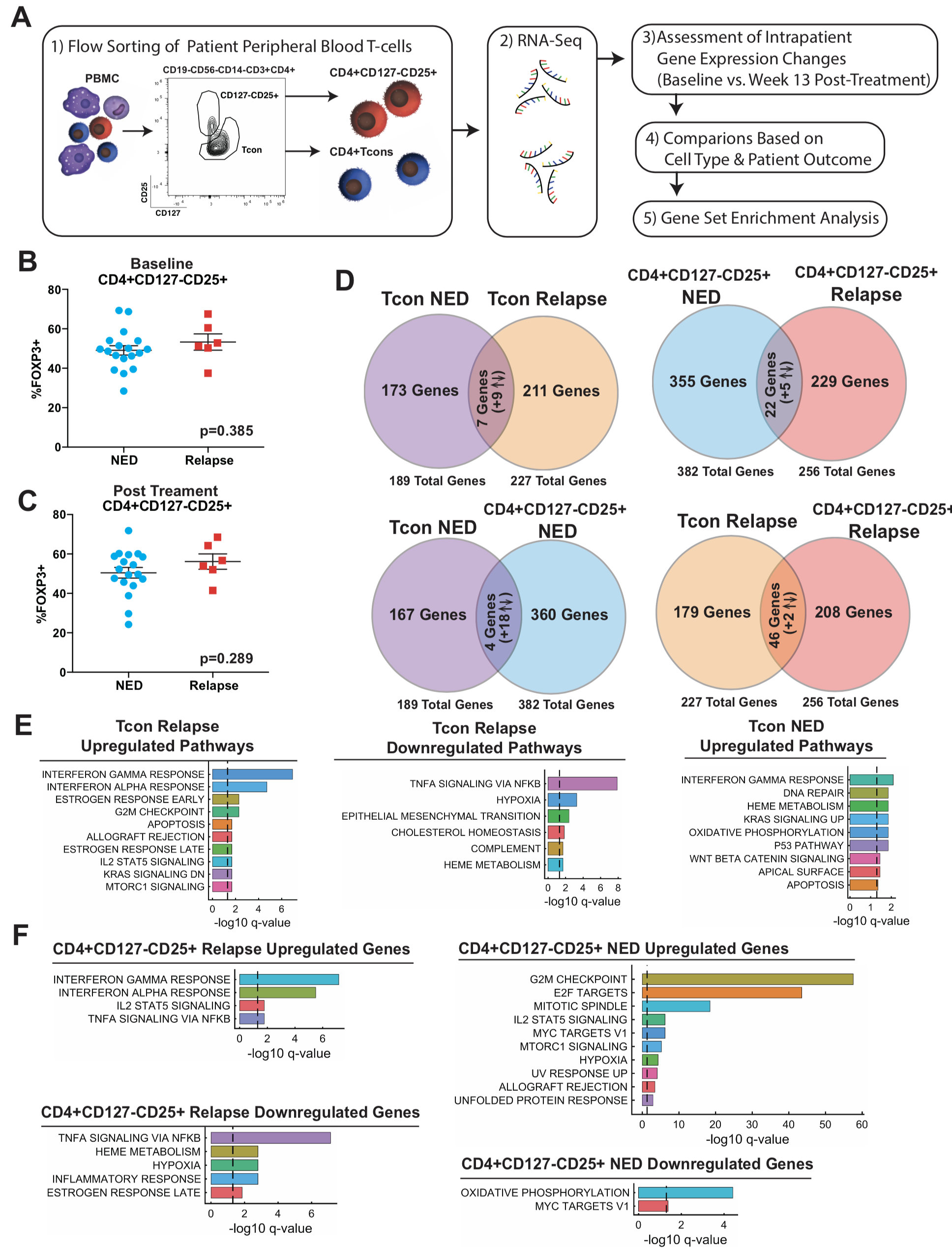
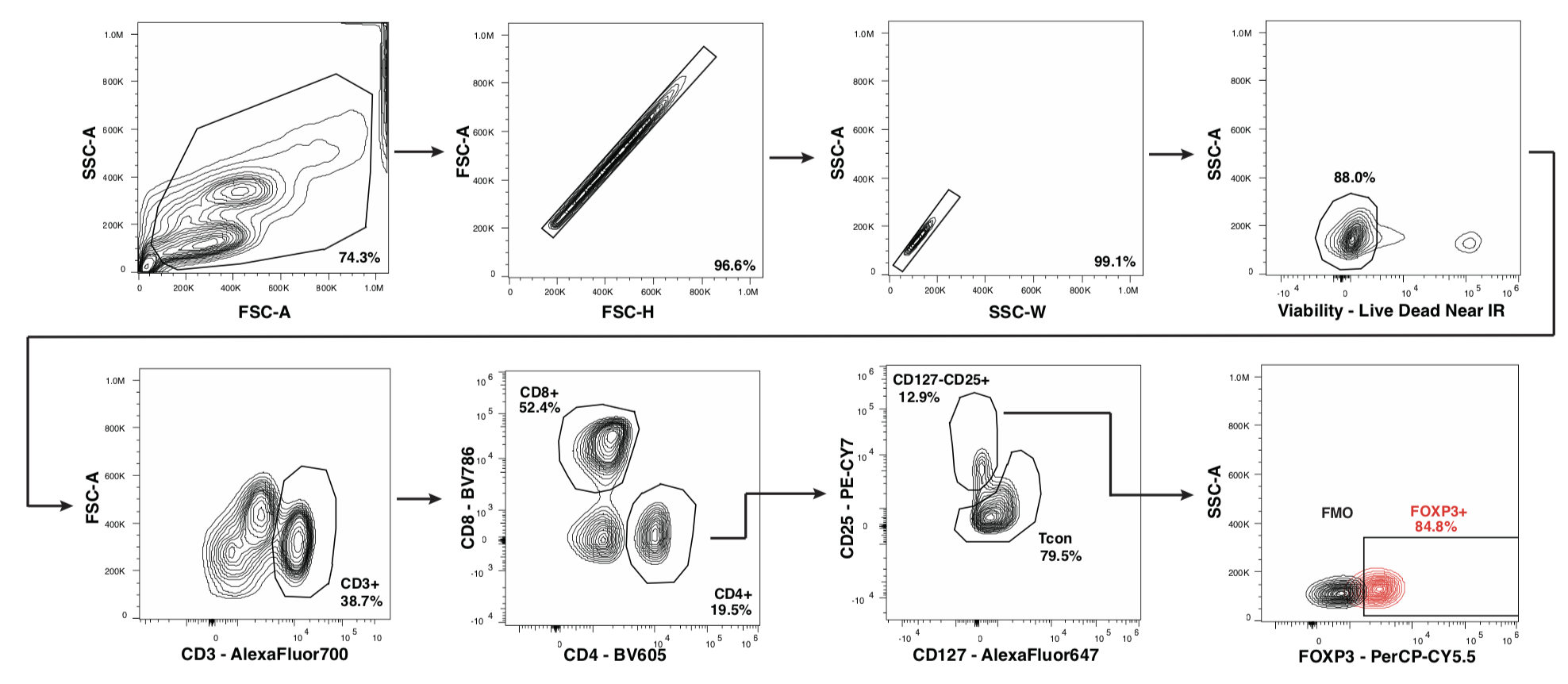
**Supplemental Figures**

**Supplemental Figure 1.**



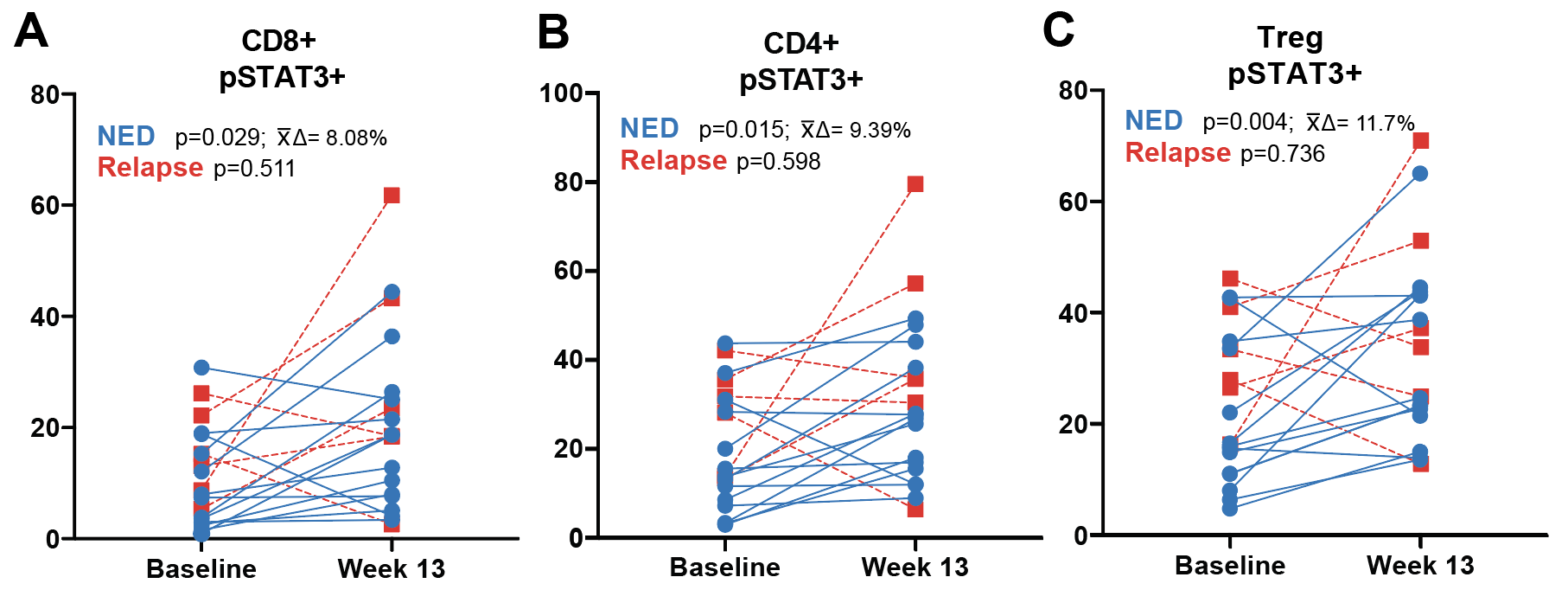
**Supplemental Figure 1. Changes in Gene Expression After Nivolumab Therapy Differ Based on Patient Outcome and T-cell Subset. (A)** Paired (baseline and week 13 after initiation of nivolumab treatment) patient PBMC were flow sorted for DAPI-CD19-CD56-CD14-CD3+CD4+CD127-/lowCD25+ T-cells and the remainder of the CD4+ population (Tcons). Cells were assessed by RNA-Seq. **(B**) Pre-treatment and **(C)** post-treatment patient CD4+CD127-/lowCD25+ peripheral blood T-cells were evaluated by intracellular flow cytometry for FOXP3 expression. **(D)** Venn diagrams with overlaps in significantly changed genes (q>0.05, comparing pre- *vs*. post samples), in NED Tcons (purple), relapse Tcons (orange), NED CD4+CD127-/lowCD25+ (blue) and relapse CD4+CD127-/lowCD25+ (red) are shown. **(E)** Tcon patient genes with significant changes (increased or decreased levels post-nivolumab) were assessed using GSEA and compared against hallmark gene sets. GSEA hallmark analysis did not return any results for downregulated NED Tcon genes and is consequently not graphed. **(F)** CD4+CD127-/lowCD25+ hallmark gene sets were likewise assessed.

**Supplemental Figure 2.**

****

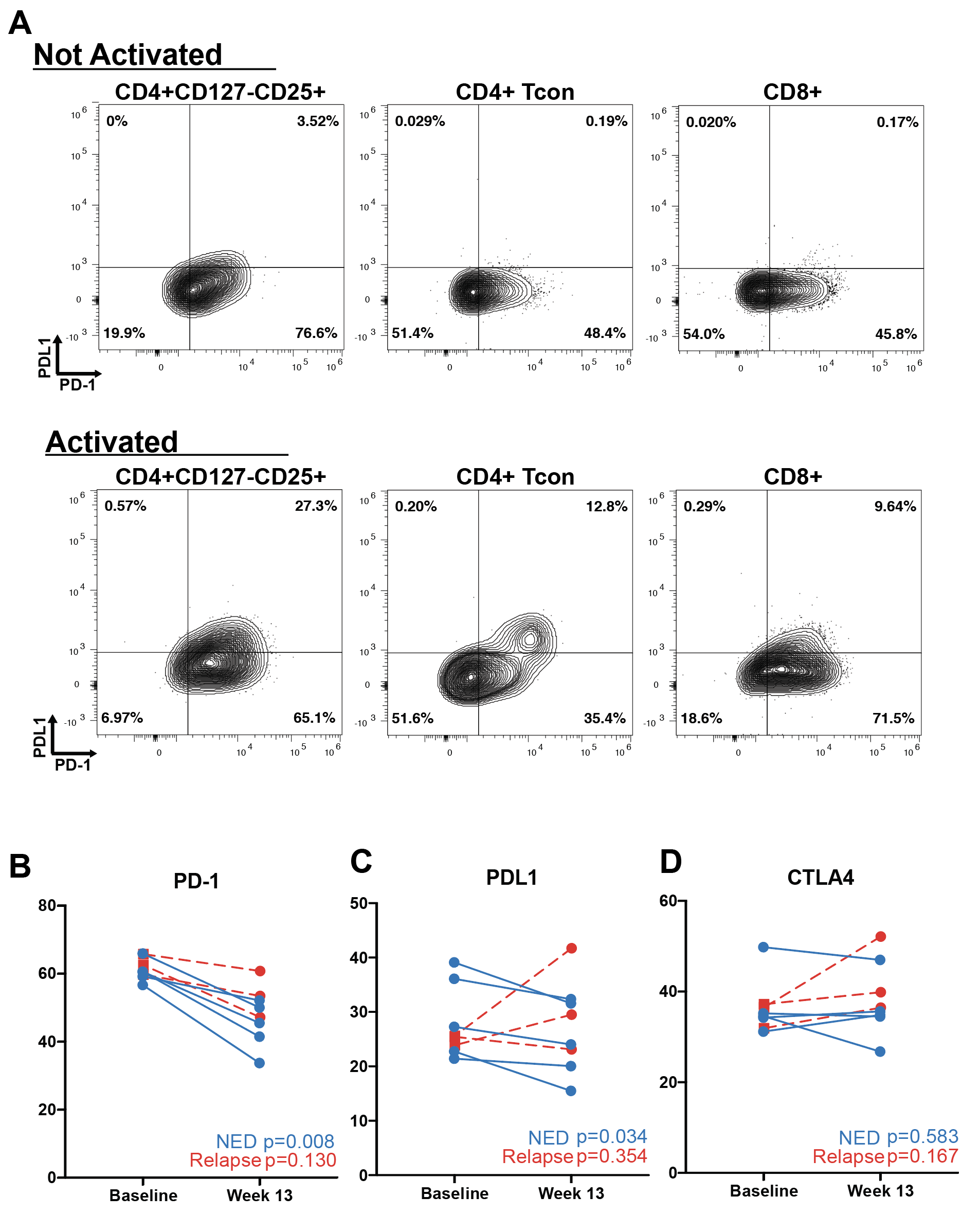
**Supplemental Figure 2. Flow Cytometry Gating Strategy.** The general gating strategy for flow cytometry and flow sorting experiments is shown. Initial FSC *vs*. SSC gate was used to gate on cells. FSC-H *v*s. FSC-A and SSC-W *vs*. SSC-A graphs were used to gate out doublets. In some experiments SSC-H *vs*. SSC-A gating was used *in lieu* of SSC-W *vs*. SSC-A. Viable cells were next gated using an amine reactive viability stain (e.g. LiveDead Near IR) or the DNA intercalating dye DAPI. CD3+ T-cells were next gated (in experiments using CD3 negative isolation, CD3 was not stained for). CD4+ *vs*. CD8+ T-cells were next gated. CD4+ were further divided into two populations: CD127-/lowCD25+ (Tregs) and the remainder of the CD4+ population (Tcons). For flow sorting, CD19, CD56 and CD8 dump channeled was used to exclude unwanted lineages. In indicated experiments, Tregs were further gated using FOXP3 expression.

**Supplemental Figure 3.**

****

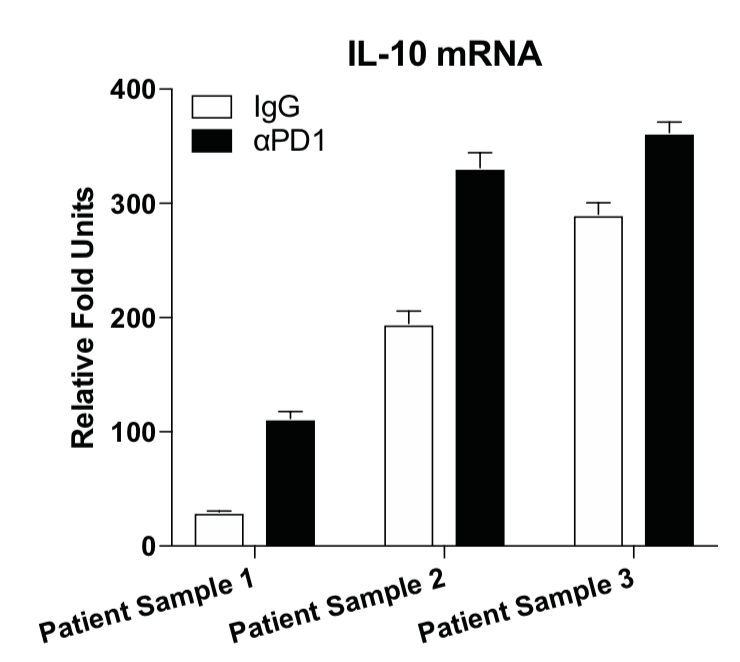
**Supplemental Figure 3. Changes in the Percentages of Patient T-cell Populations Expressing Phosphorylated STAT3.** PairedPBMC samples (baseline and week 13 after initiation of nivolumab treatment) from resected patients treated with adjuvant nivolumab were assessed by flow cytometry and percentages of **(A)** CD8+T-cells, **(B)** CD4+ Tcons and **(C)** Tregs expressing pSTAT3 were assessed. Blue lines connect paired NED patient samples and red, dotted lines connect relapsing patient samples. P-values are reported in the graphs along with average change in means (∆x̄) for NED patients.

**Supplemental Figure 4.**

****

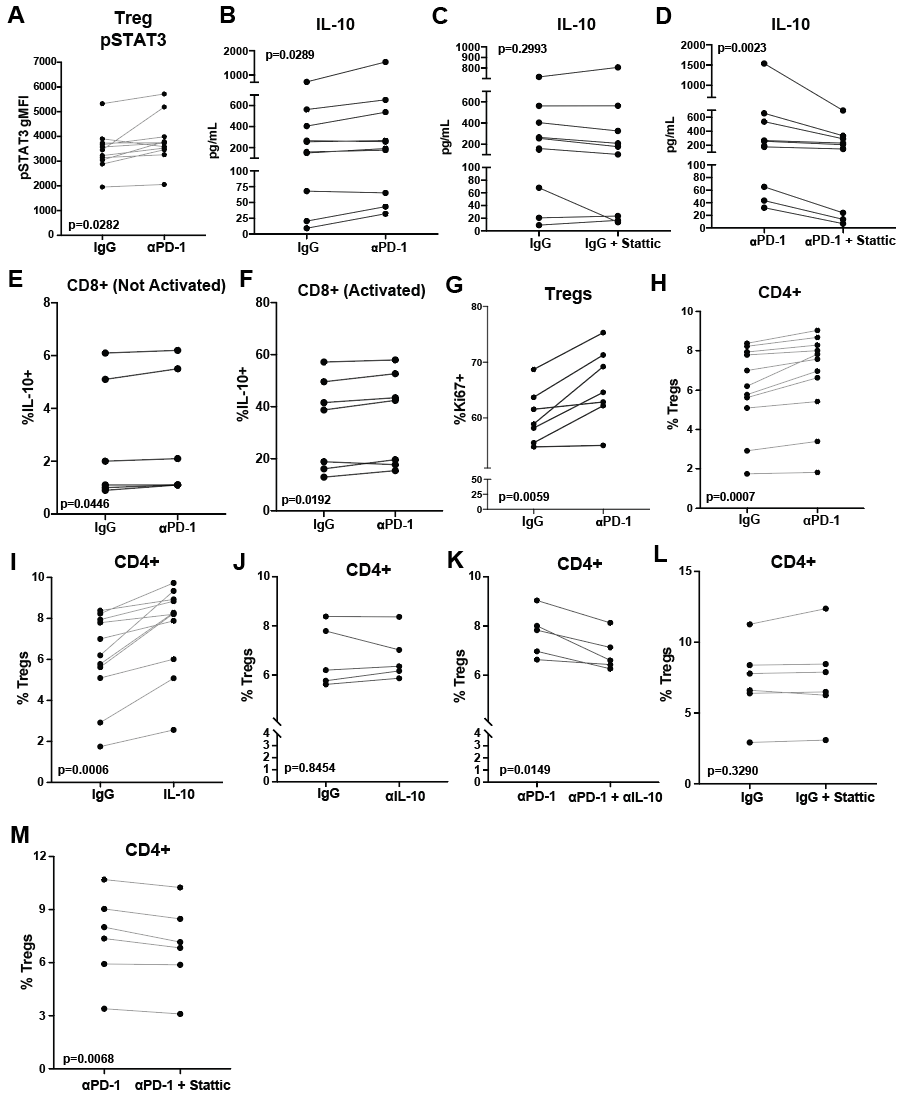
**Supplemental Figure 4. T-cells express PDL1. (A)** T-cell populations were evaluated by flow cytometry for expression of PD-1 and PDL1 without (top graphs) or with the addition of CD3/CD28 Dynabead activation (bottom graphs). **(B-D)** Patient CD4+CD127-CD25+ T-cells were evaluated for expression of indicated co-inhibitory markers. Blue lines connect paired NED patient samples and red, dotted lines connect relapsing patient samples. P-values from ratio paired T-tests are reported in the graphs.

**Supplemental Figure 5.**

****

**Supplemental Figure 5. PD-1 Blockade Increases *IL-10* Expression *in Vitro.*** CD3+ T-cells isolated from three baseline patient PBMC samples were cultured overnight with IgG (white bars) or αPD-1 (5ug/mL) (black bars) then activated for six hours using CD3/CD28 Dynabeads. *IL-10* gene expression was measured by qRT-PCR. Fold units were calculated relative to 18S rRNA using ∆CT and normalized to IgG non-activated controls (∆∆CT). Error bars are SEM of technical triplicates.

**Supplemental Figure 6.**

****

**Supplemental Figure 6. Paired Analyses of *In Vitro* Experiments. (A)** Isolated baseline patient CD3+ T-cells were cultured with the addition of αPD-1 (5ug/mL) for 48 hours. Tregs were evaluated by flow cytometry for pSTAT3 expression levels. Paired analyses of samples assessed over seven independent experiments are shown. **(B-D)** Isolated baseline patient CD3+ T-cells were cultured with the addition of indicated treatments for two hours and subsequently activated by CD3/CD28 Dynabeads. After 48 or 72 hours, culture supernatants were assessed for IL-10. Paired analyses of samples assessed over five independent experiments are shown. **(E-F)** Isolated baseline patient CD3+ T-cells were cultured with IgG or αPD-1 (5ug/mL) **(E)** without or **(F)** with CD3/CD28 Dynabead activation. After 72 hours, cells were assessed by intracellular flow cytometry for CD8+ IL-10 production. Paired samples were assessed over two independent experiments. **(G)** Isolated baseline patient CD3+ T-cells were cultured with the addition of indicated treatments overnight and subsequently activated by CD3/CD28 Dynabeads. After 48 hours, CD4+CD127-CD25+FOXP3+ cells were assessed for Ki67 expression. Paired analyses were assessed over two independent experiments. **(H-M)** Isolated baseline patient CD3+ T-cells were cultured with the indicated treatments for 48 hours and assessed by flow cytometry for Tregs as a percentage of the CD4+ population. For all plots, pairs are based on pre- and post- *in vitro* treatment of the same patient sample. Paired samples were assessed in three to seven independent experiments. Accompanying p-values, determined by paired t-tests, are shown in each graph.