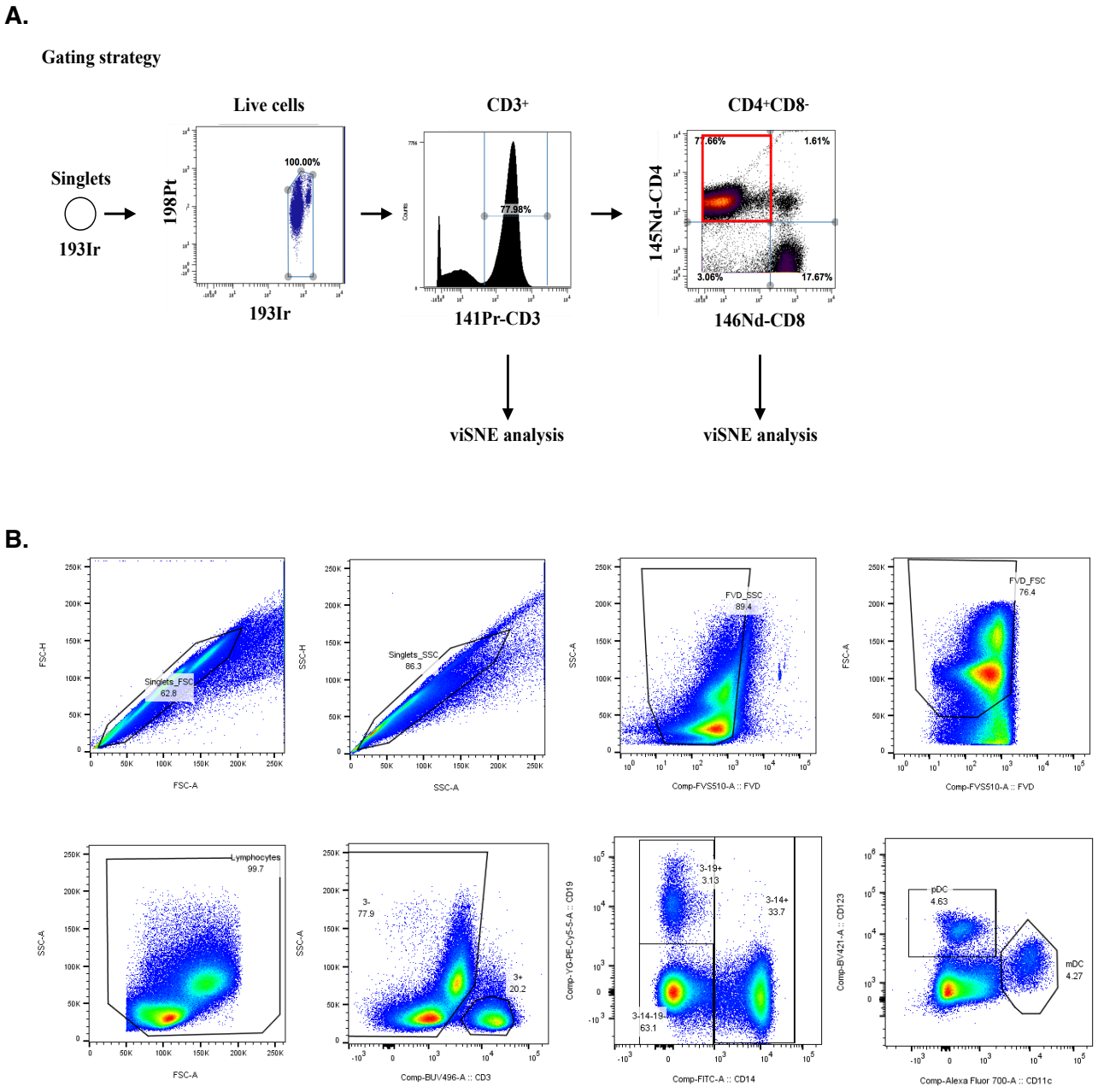
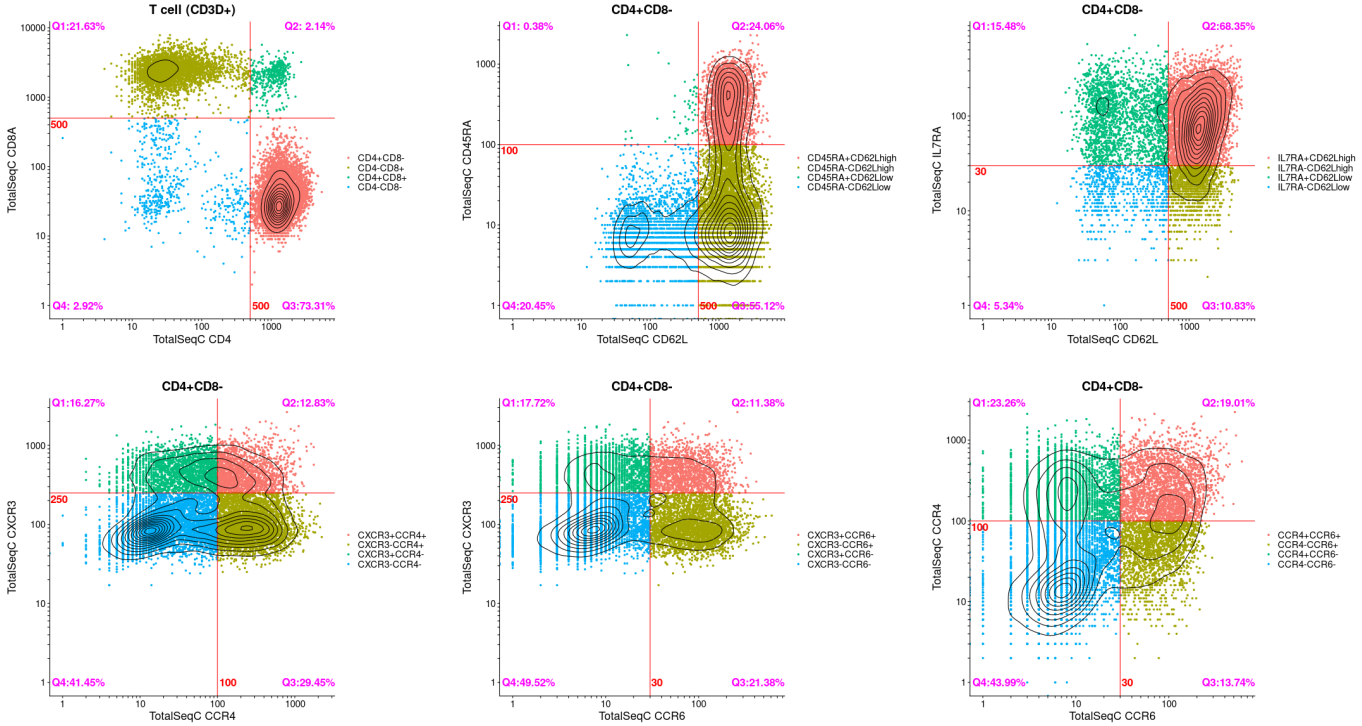


Gating strategies for mass cytometry analysis (**A**) and flow cytometry analysis (**B**) are shown.

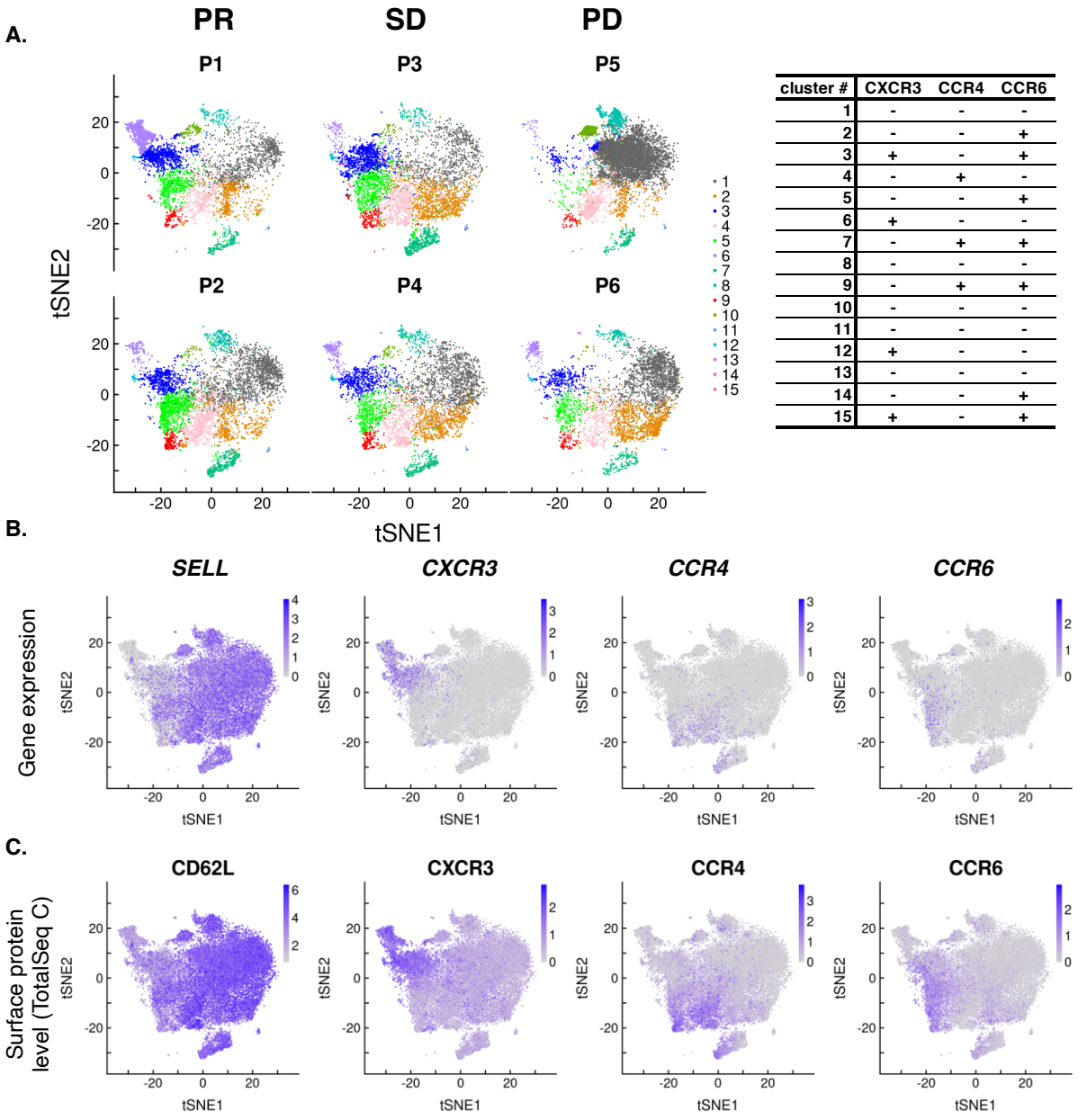
Supplementary Figure 1

Product information of TotalSeq™-C used in our study
TotalSeq™-C0072 anti-human CD4 Antibody
TotalSeq™-C0080 anti-human CD8 Antibody
TotalSeq™-C0147 anti-human CD62L Antibody
TotalSeq™-C0063 anti-human CD45RA Antibody
TotalSeq™-C0140 anti-human CD183 (CXCR3) Antibody
TotalSeq™-C0071 anti-human CD194 (CCR4) Antibody
TotalSeq™-C0143 anti-human CD196 (CCR6) Antibody
TotalSeq™-C0154 anti-human CD27 Antibody
TotalSeq™-C0390 anti-human CD127 IL-7Rα Antibody
TotalSeq™-C090 anti-mouse IgG1, κ Antibody



Gating strategies of scRNAseq analysis with TotalSeqC technique are shown.

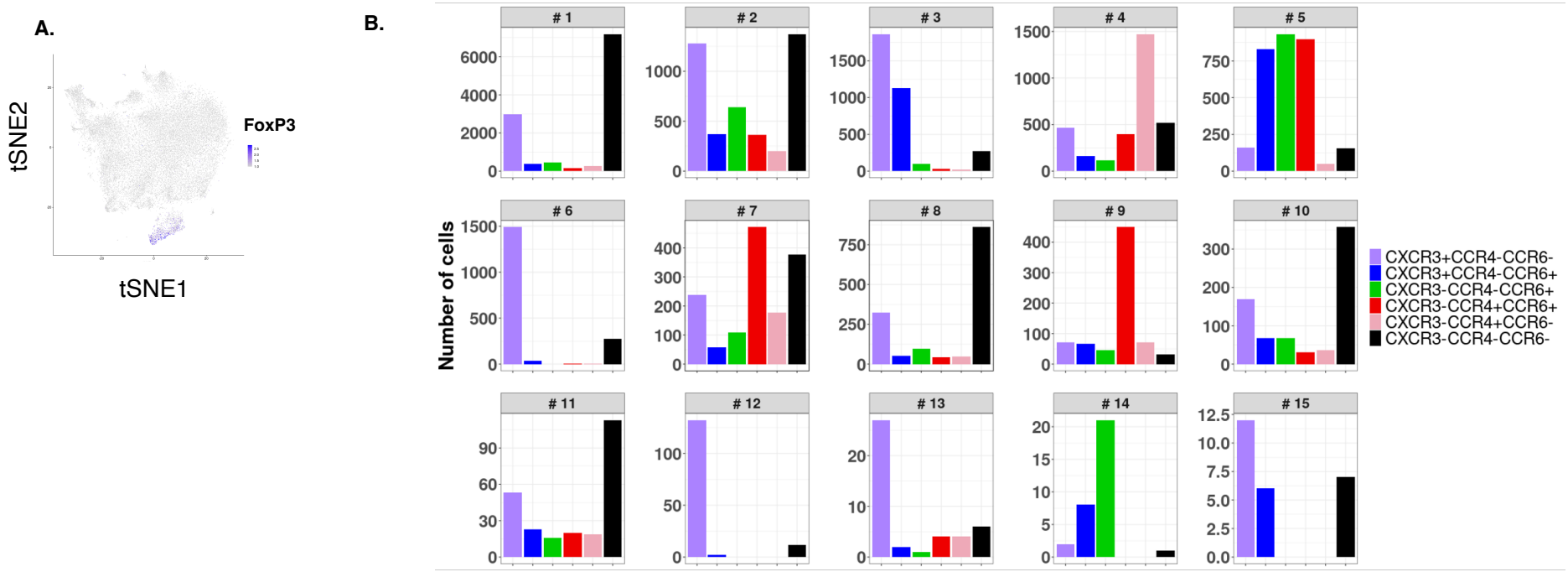
Supplementary Figure 2



cluster #	CXCR3	CCR4	CCR6
1	-	-	-
2	-	-	+
3	+	-	+
4	-	+	-
5	-	-	+
6	+	-	-
7	-	+	+
8	-	-	-
9	-	+	+
10	-	-	-
11	-	-	-
12	+	-	-
13	-	-	-
14	-	-	+
15	+	-	+

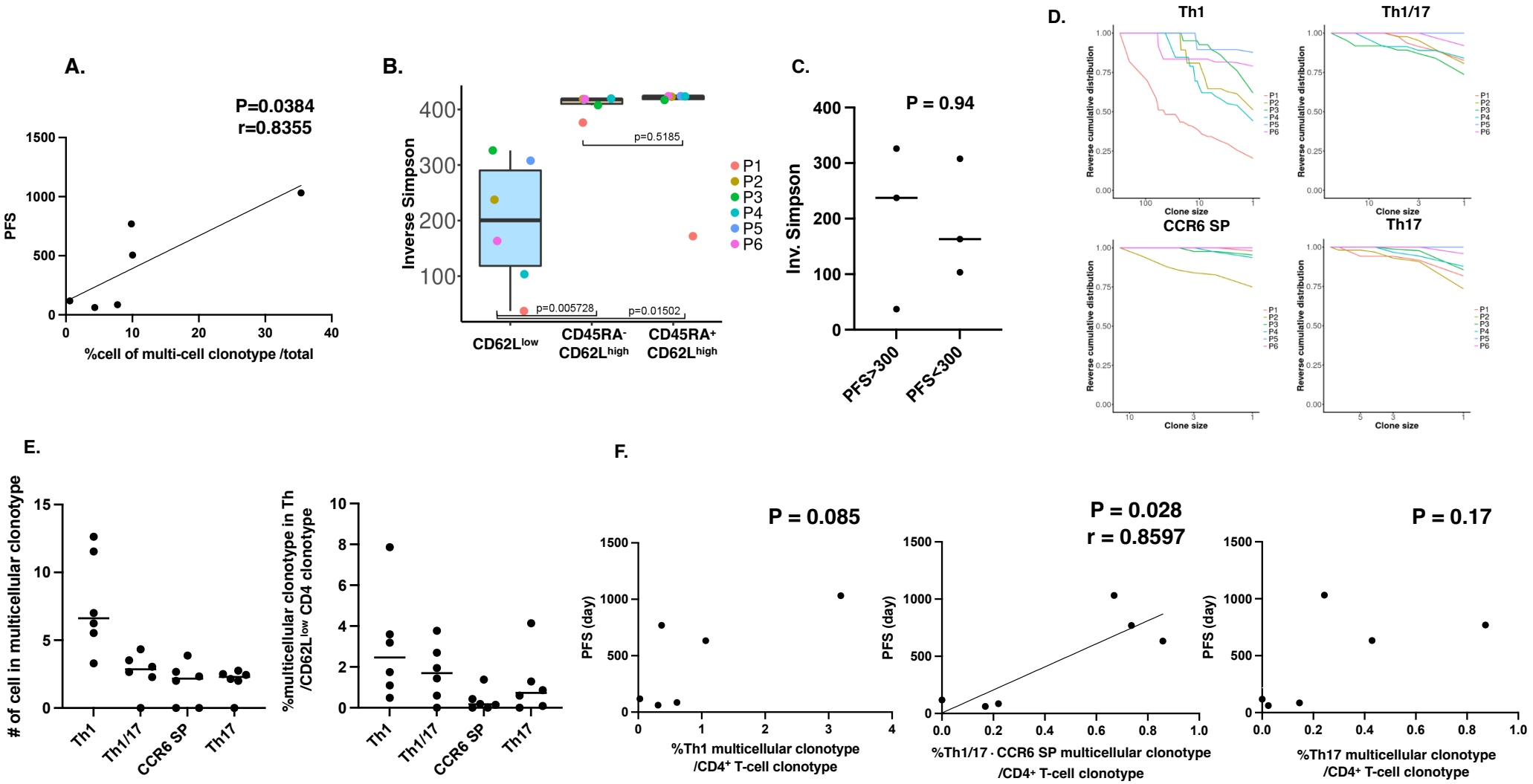
A. Unsupervised clustering results for each patient based on scRNAseq analysis.
B, C: Mapping of gene and protein level expression of CD62L, CXCR3, CCR4, and CCR6 that were used to annotate T-cell clusters on the unsupervised clustering plot.

Supplementary Figure 3



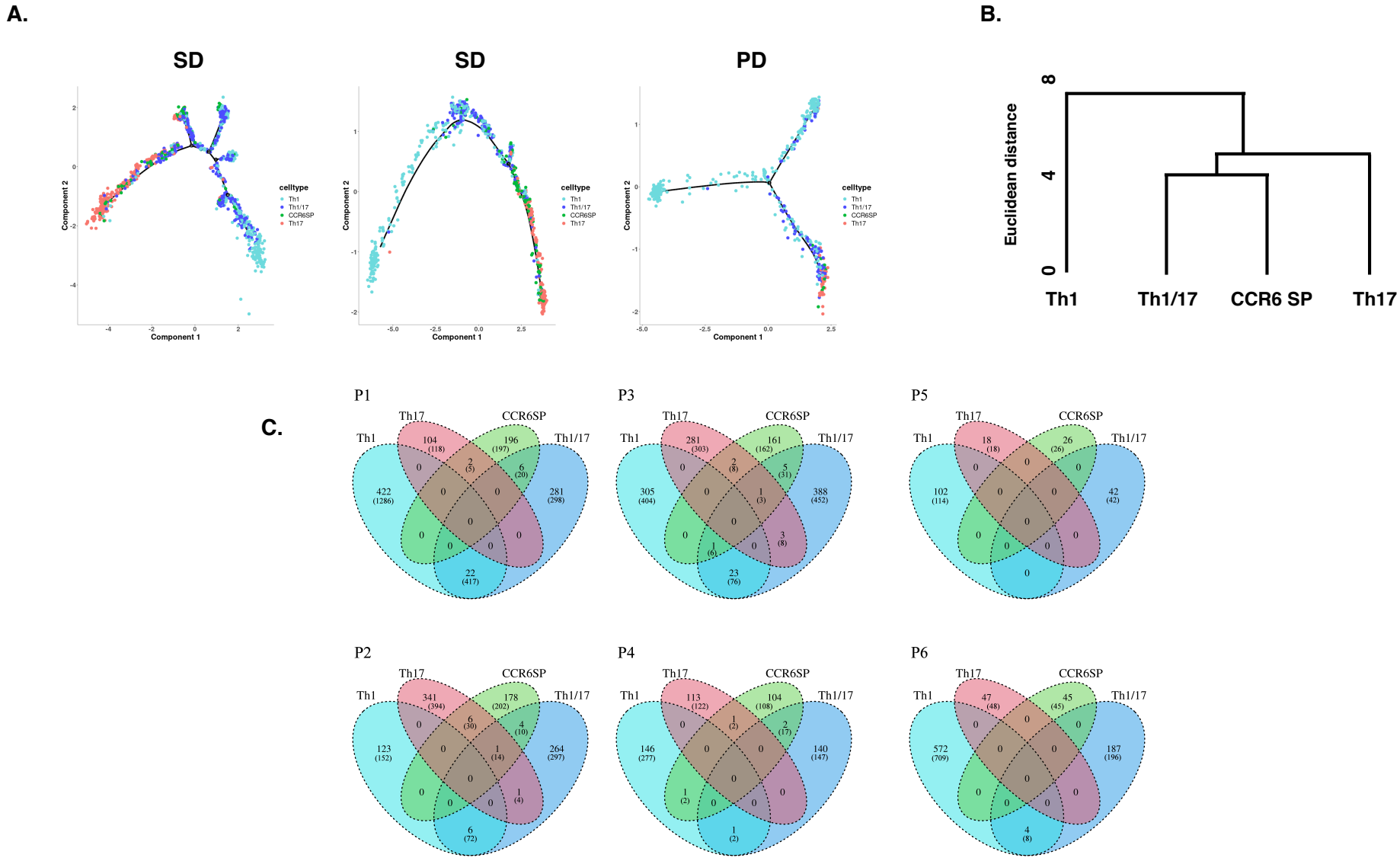
A: Cell-level gene expression of FoxP3 in tSNE analysis is shown.
B: Cell-level expression pattens of CXCR3, CCR4, and CCR6 in 15 clusters obtained by unsupervised clustering.

Supplementary Figure 4



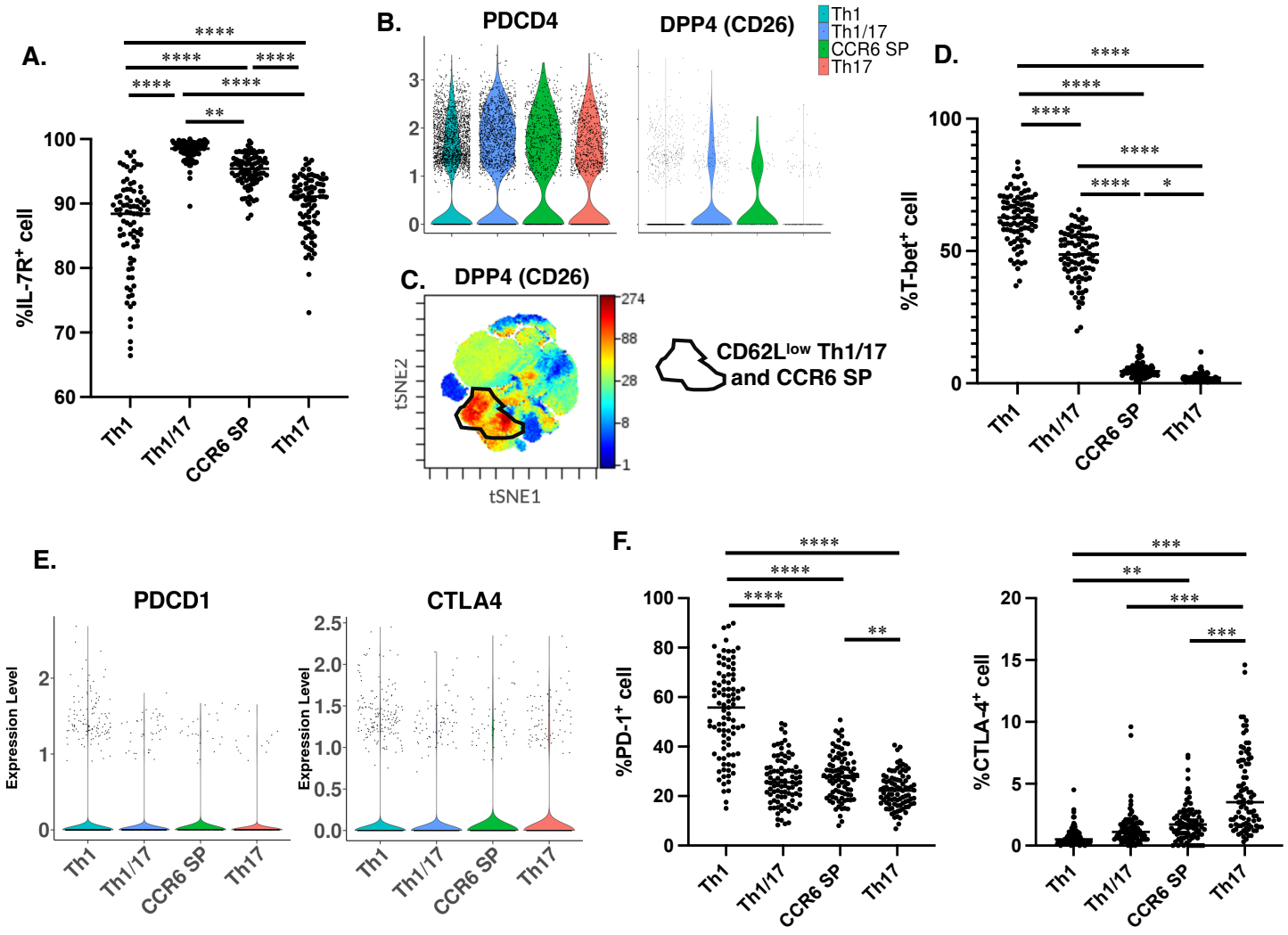
A: Correlation between the T-cell percentage of multicellular clonotypes and progression-free survival (PFS) after pembrolizumab therapy for previously untreated NSCLC patients.
B: Clonotype diversity of CD62L^{low}, CD45RA-CD62L^{high}, CD45RA^{+}CD62L^{high} CD4^{+} clusters for six patient.
C: Comparison of TCR diversity index for PFS>300 days and PFS<300 days.
D: Reverse cumulative distribution of clone size per Th cluster belonging to the CD62L^{low} CD4^{+} T-cell subpopulation.
E: Number of cells belonging to multicellular clonotypes and number of multicellular clonotypes per Th cluster belonging to CD62L^{low} CD4^{+} T cell subpopulation.
F: Correlation between the percentage of multicellular clonotypes per Th cluster and PFS.

Supplementary Figure 5



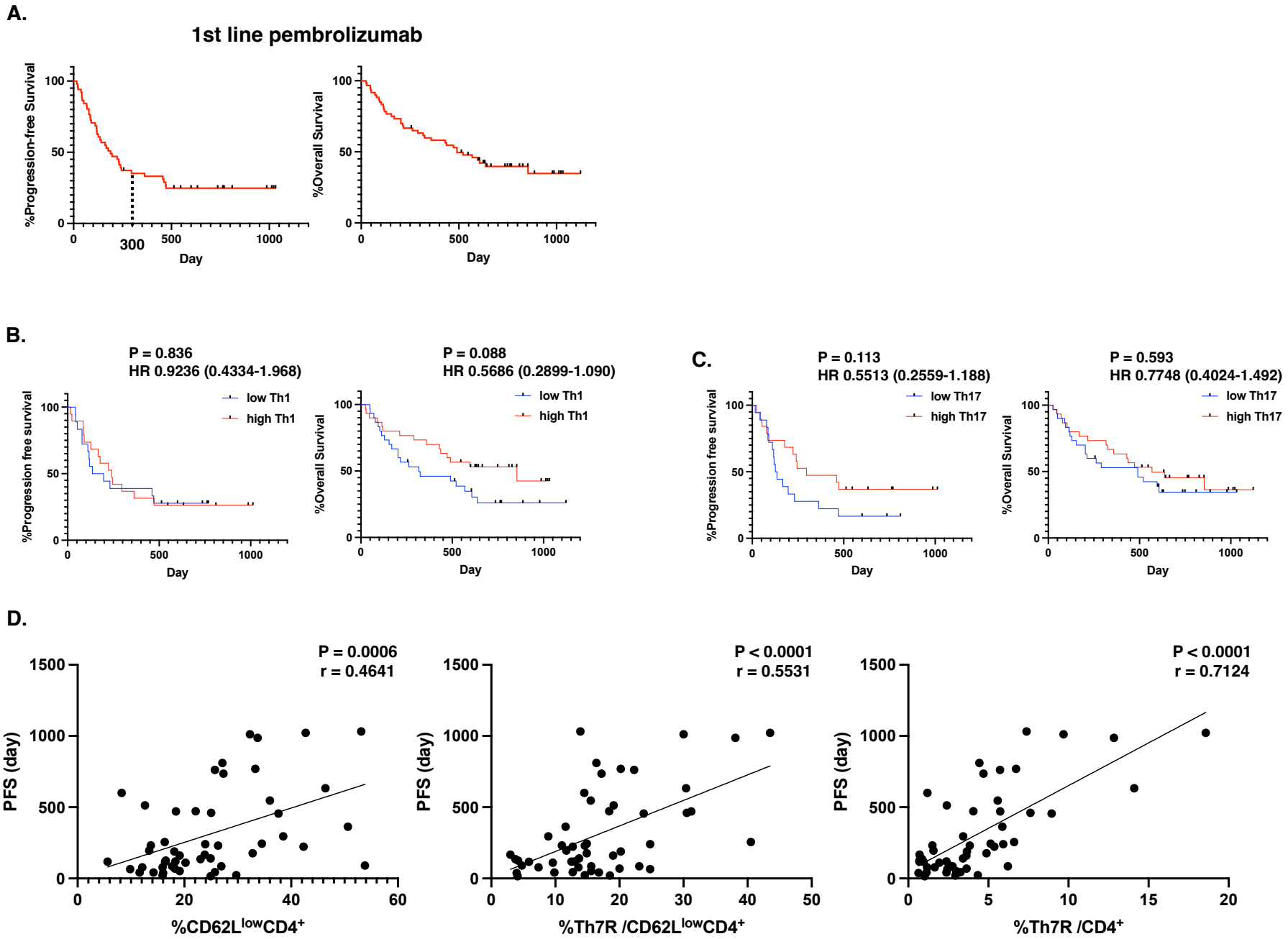
A: The results of pseudo-time analysis calculated from differentially expressed genes between Th1-Th17 clusters of each patient (P3 (SD), P4 (SD), P6 (PD)). For the P5 (PD) patient, this analysis could not be done because the number of cells belonged to CD62L^{low} was too small.
B: Hierarchical clustering results based on the gene expression profiles of signature genes revealed by one-way ANOVA of the four clusters in CD62L^{low} CD4^{+} T cell subpopulation. Details on differentially expressed genes are shown in Table S8.
C: Th1/17, CCR6 SP, and Th17) of individual patients are shown. Numbers represent the number of clonotypes and the numbers with bracket represent the number of cells included in that category.

Supplementary Figure 6



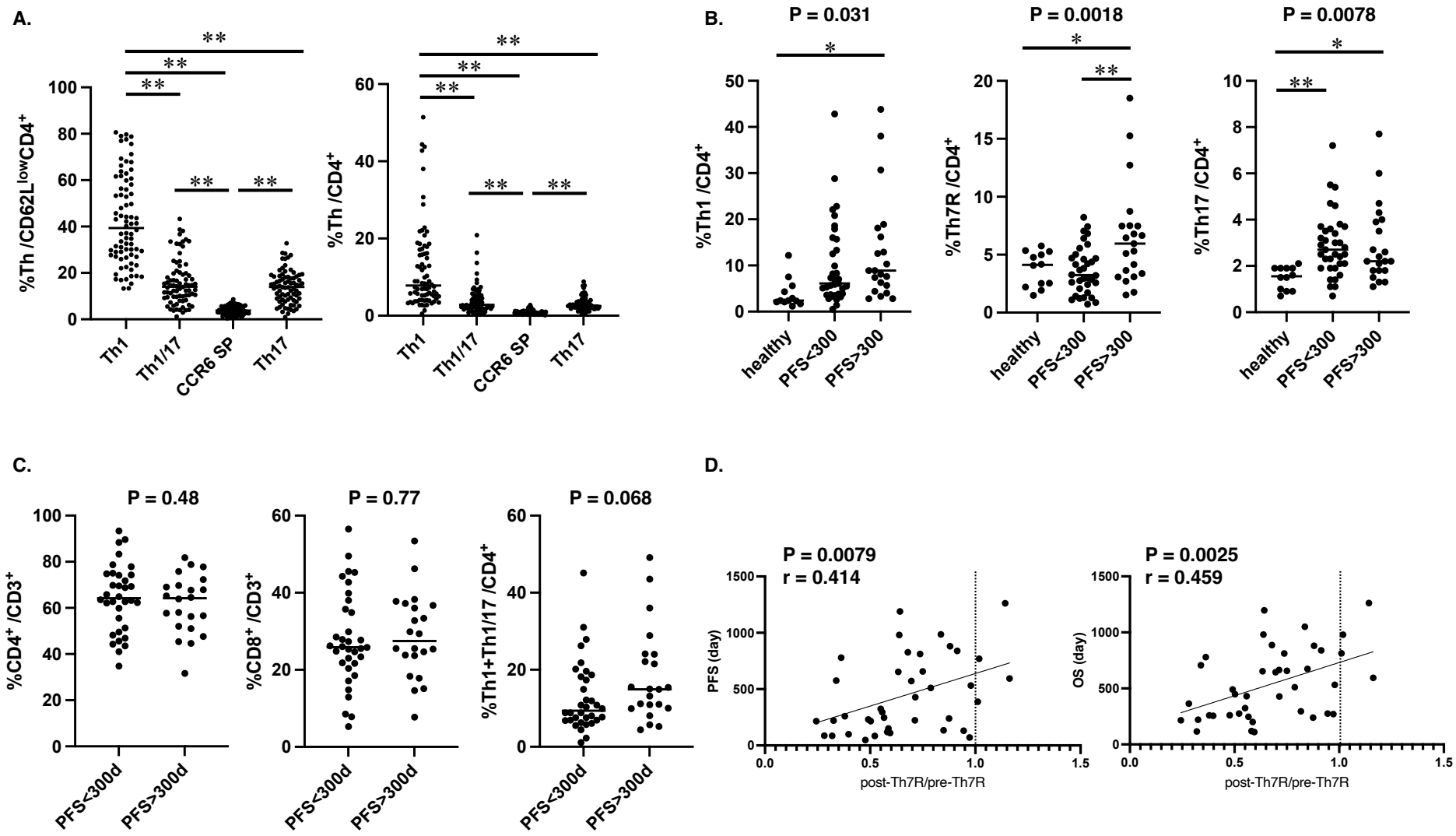
A: The percentages of IL-7R$^+$ cells in gated CD62L^{low} Th1, Th1/17, CCR6 SP, and Th17 clusters of NSCLC patients are shown based on CyTOF analysis.
B: *PDCD4* and *DPP4* gene expression in CD62L^{low} Th1, Th1/17, CCR6 SP, and Th17 clusters.
C: DPP4 expression is shown based on CyTOF analysis. The black line indicates CD62L^{low} Th1/17 and CCR6 SP region.
D: The percentages of T-bet$^+$ cell in gated CD62L^{low} Th1, Th1/17, CCR6 SP, and Th17 clusters of NSCLC patients are shown based on CyTOF analysis.
E: *PDCD1* and *CTLA-4* gene expression in CD62L^{low} Th1, Th1/17, CCR6 SP, and Th17 clusters.
F: PD-1$^+$, and CTLA-4$^+$ cells in gated CD62L^{low} Th1, Th1/17, CCR6 SP, and Th17 clusters of NSCLC patients are shown based on CyTOF analysis.
G: Correlation analysis between the percentages of CD62L^{low} Th1/17 cells and the percentages of CD62L^{low} CCR6 SP, Th17, or Th1 cells in the peripheral blood of NSCLC patients.
Statistical analysis in A, D, and F was performed by one-way ANOVA with post-hoc Tukey's multiple comparison test. **** indicates that $P < 0.0001$, *** indicates that $P < 0.001$, ** indicates that $P < 0.01$, and * indicates that $P < 0.05$.

Supplementary Figure 7



A: Kaplan-Meier analysis of PFS and OS in the 1st line pembrolizumab cohort (n=60).
B: Kaplan-Meier analysis of PFS and OS divided by the median Th1 in the 1st line pembrolizumab cohort. No significant differences were observed.
C: Kaplan-Meier analysis of PFS and OS divided by the median Th17 in the 1st line pembrolizumab cohort. No significant differences were observed.
D: Correlation of PFS with the $CD4^+$ T cell-based $CD62^{Low}$ cell percentage, the $CD62L^{low}$ $CD4^+$ T cell-based Th7R percentage, and the $CD4^+$ T cell-based Th7R percentage in the 1st line pembrolizumab cohort.

Supplementary Figure 8



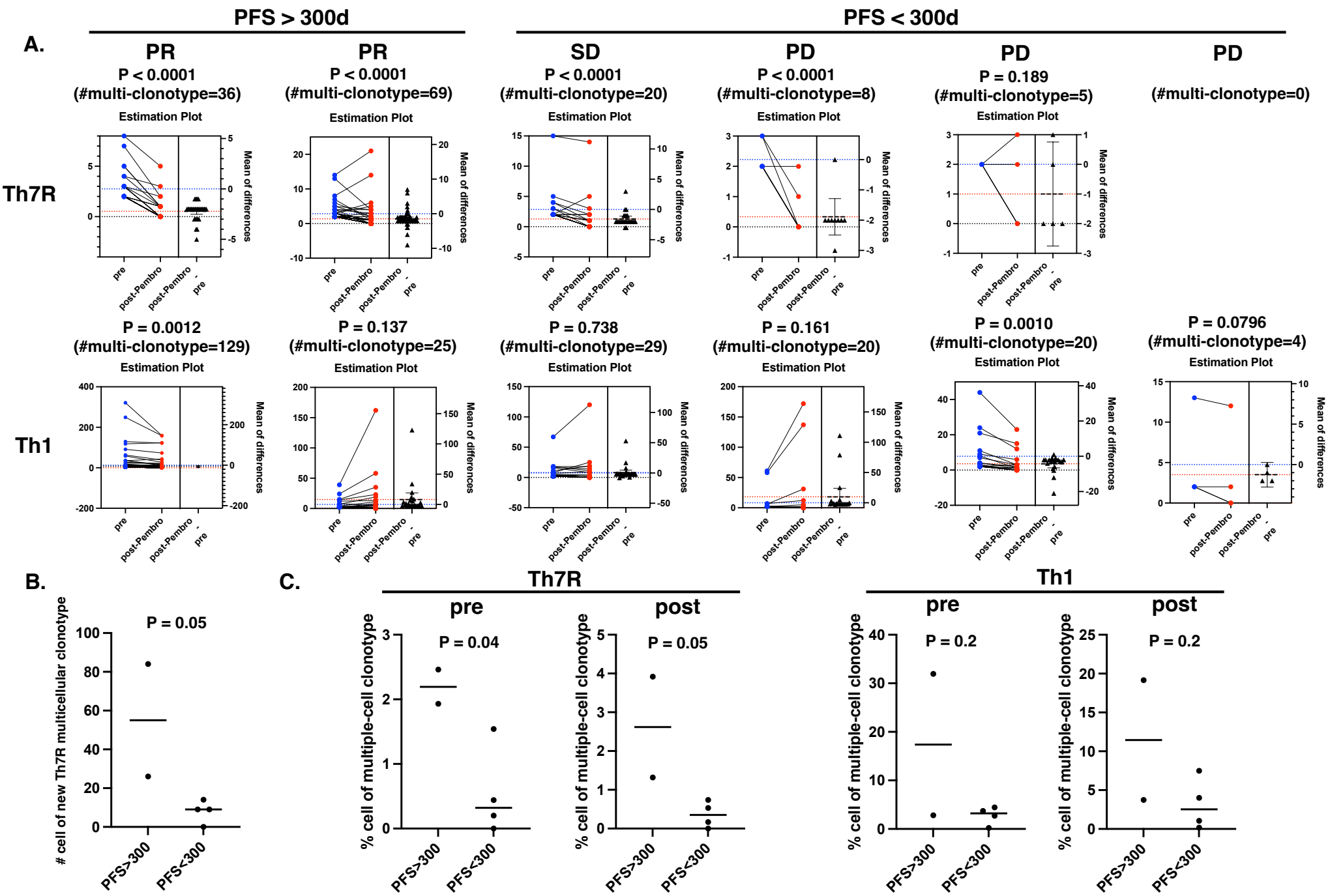
A: The percentage of Th1, Th1/17, CCR6 SP, and Th17 based on CD62L^{low} CD4^{+} T cells, and the percentage of each Th cluster based on total CD4^{+} T cells of the first pembrollizumab-treated lung cancer cohort.

B: The percentages of Th1, Th7R, and Th17 in the PFS<300 days and PFS>300 days groups in the first pembrolizumab-treated lung cancer cohort and healthy volunteers (n=12) are presented. *; P < 0.05, **; P<0.01, one-way ANNOVA with Turkey's multiple comparisons test

C: The percentages of CD4^{+}, CD8^{+}, and IFNγ producing Th (Th1 + Th1/17) in the PFS<300 days and PFS>300 days groups in the first pembrolizumab-treated lung cancer cohort are presented. P-values were obtained using the unpaired Student t-test.

D: The correlation between the ratio of post-treatment Th7R percentage to pre-treatment Th7R percentage and PFS or OS.

Supplementary Figure 9



A: The number of cells belonging to the Th7R and Th1 multicellular types before pembrolizumab therapy was compared before and after therapy (n=6). P-values were obtained using the paired Student t-test.

B: The number of cells belonging to the Th7R multicellular clonotypes that were singletone or not detected before pembrolizumab treatment but newly detected after treatment as multicellular clonotype was compared between patients with PFS > 300 days and < 300 days. P-vales were obtained using the unpaired Student t-test.

C: The percentages of cells belonging to the Th7R and Th1 multicellular types before and after pembrolizumab treatment were compared between patients with PFS >300 days and those with PFS <300 days. P-values were obtained using the unpaired Student t-test.

Supplementary Figure 10