Figure S1. Histogram depicting distribution (n=31) of the interval (days) between 1st and 2nd pembrolizumab administration.

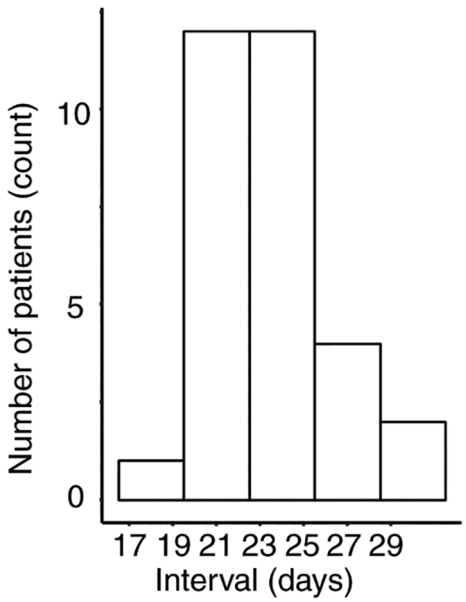

Figure S2. Continued.

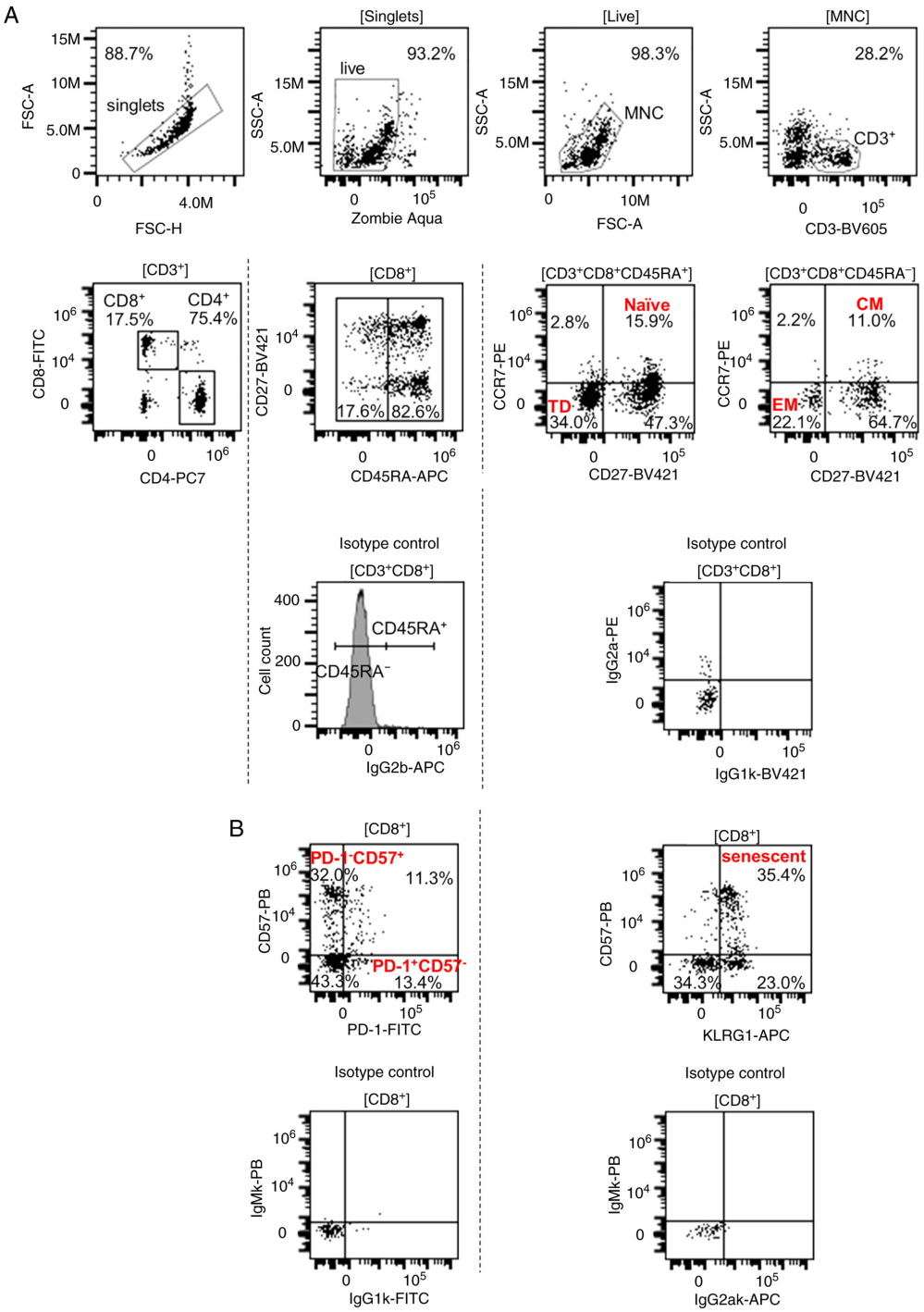

Figure S2. Continued.

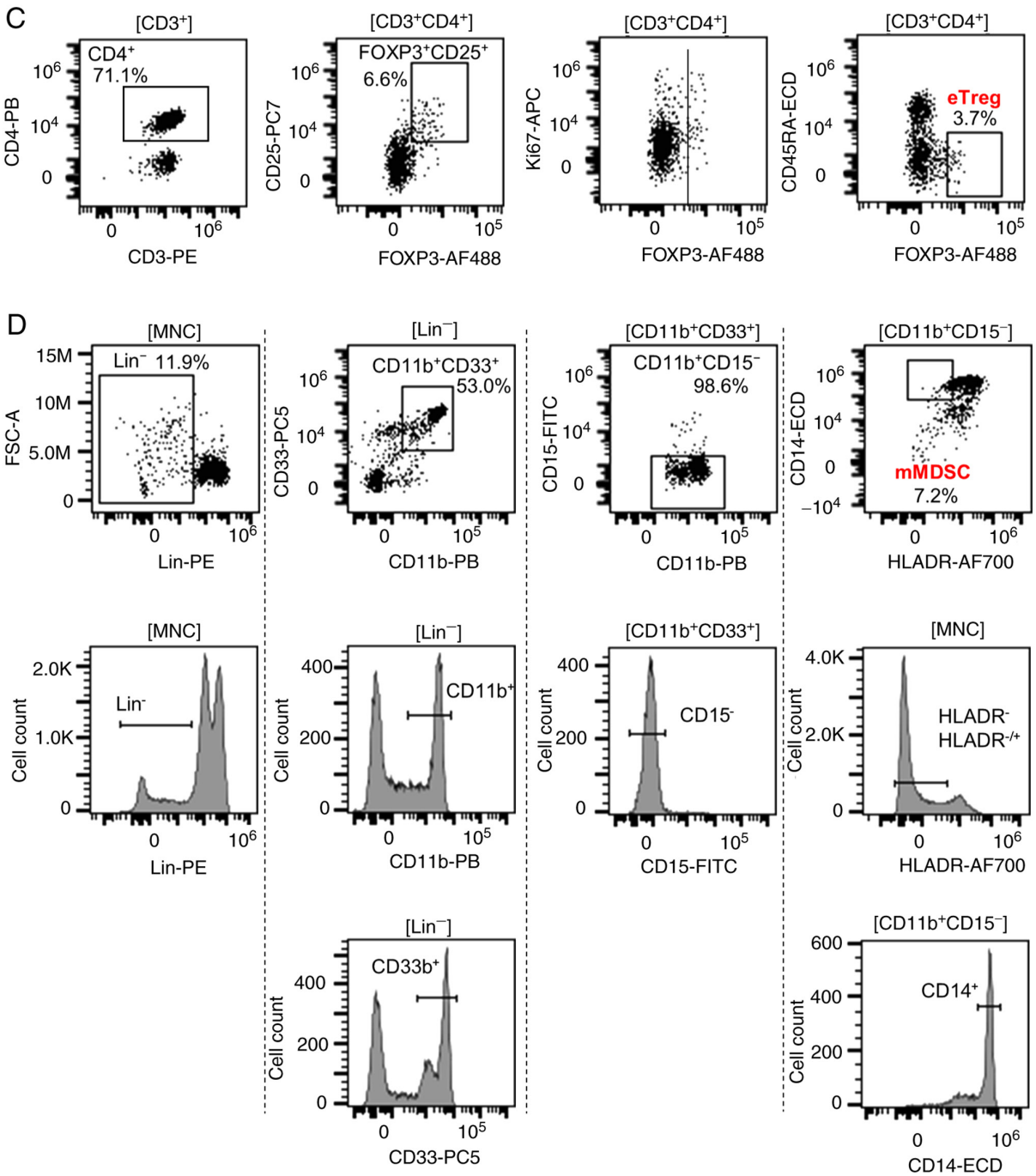

Figure S2. Continued.

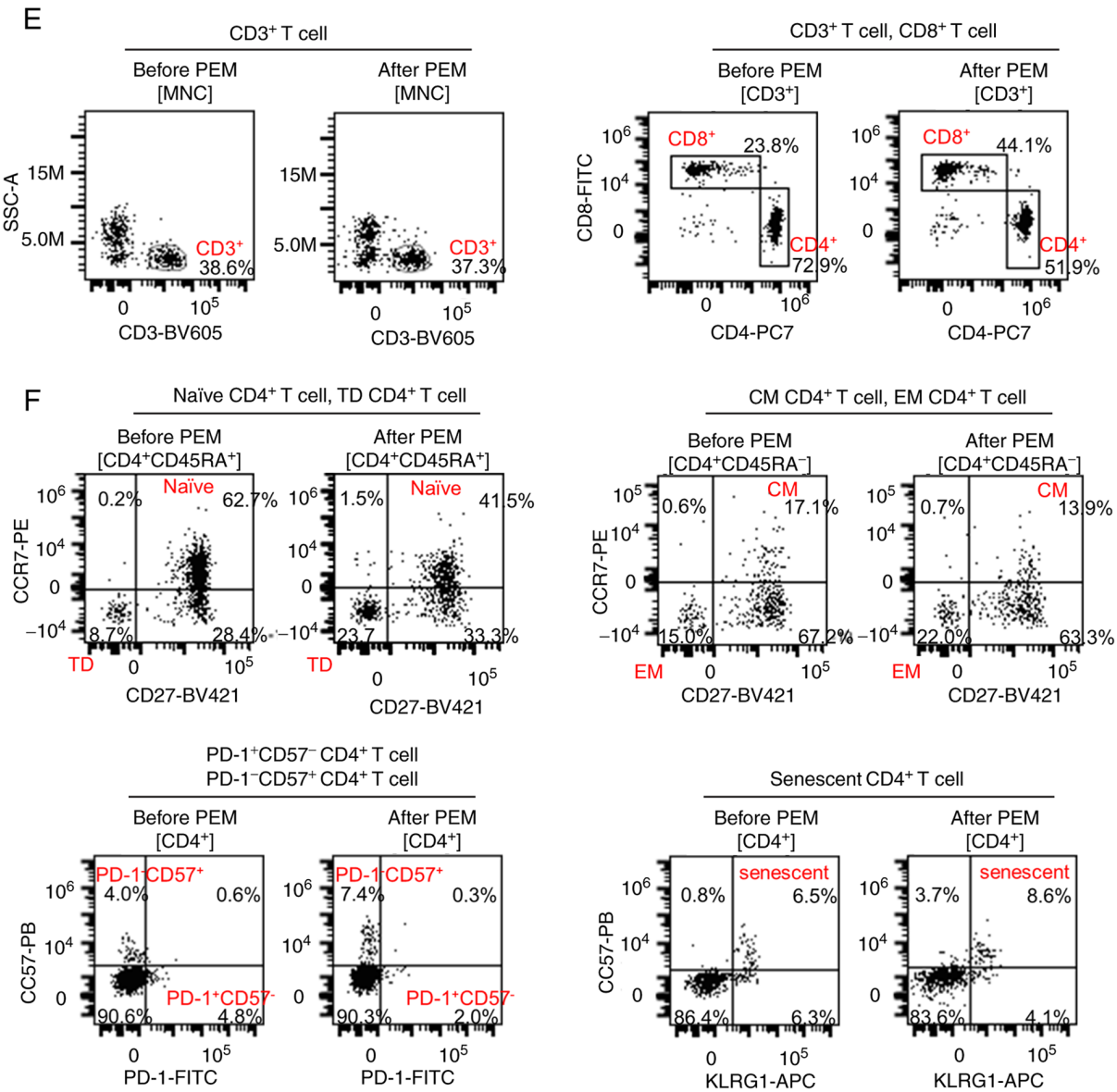

Figure S2. Continued.

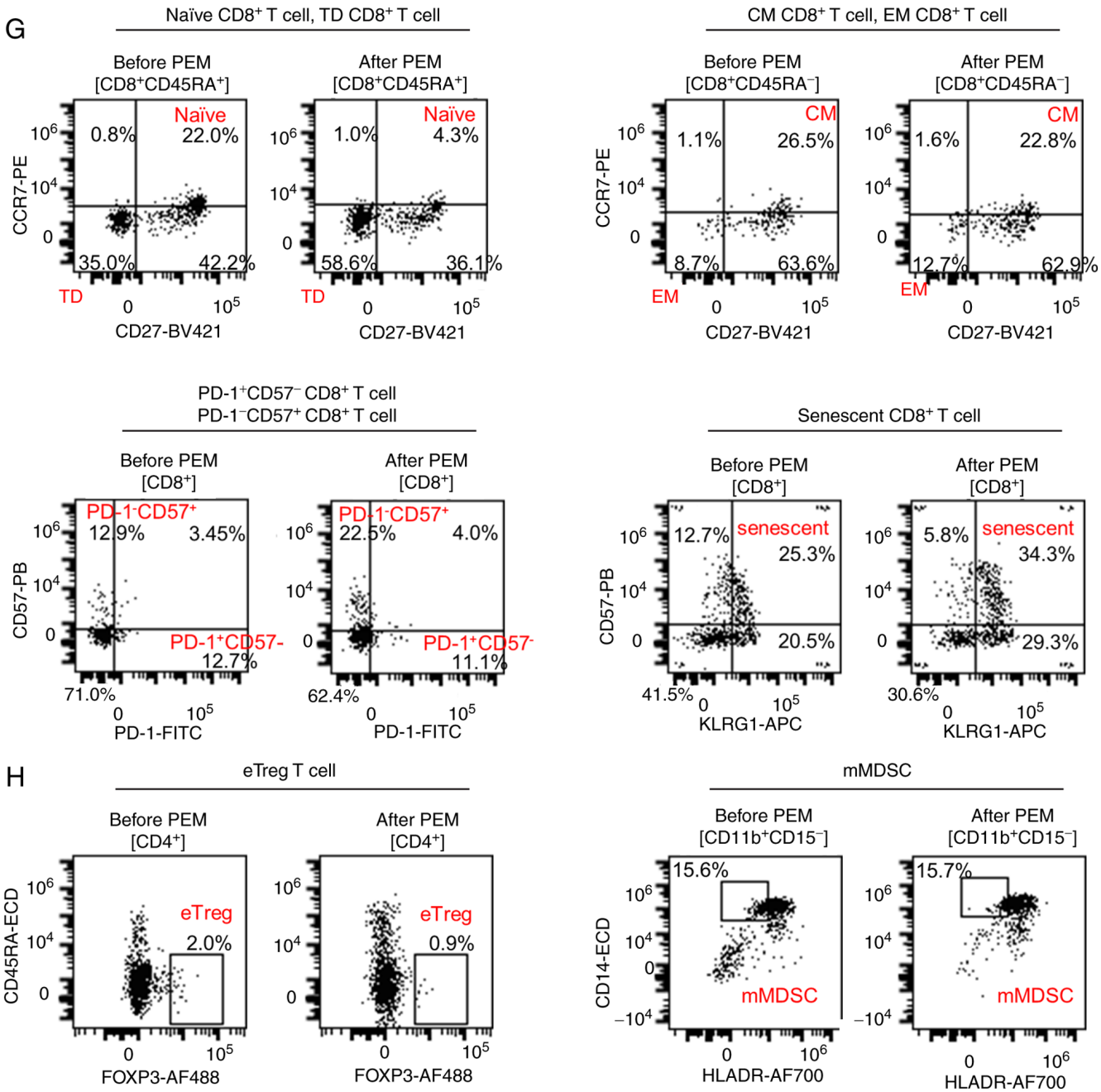

Figure S2. Gating strategies for T cells, eTregs and mMDSCs. (A) Gating strategy for CM CD4$^+$ or CM CD8$^+$, EM CD4$^+$ or EM CD8$^+$, naïve CD4$^+$ or naïve CD8$^+$, TD CD4$^+$ or TD CD8$^+$ cells. (B) Gating strategy for KLRG1$^+$CD57$^+$ (senescent) CD4$^+$ and CD8$^+$ T cells. (C) Gating strategy for PD-1$^+$CD57$^-$ (exhausted fraction, not senescent) CD4$^+$ or PD-1$^+$CD57$^-$ CD8$^+$, PD-1$^-$CD57$^+$ (senescent, not exhausted) CD4$^+$ or PD-1$^-$CD57$^+$ CD8$^+$ T cells. (D) Gating strategy for lineage marker (CD3, CD16, CD19, CD20, CD56)-negative CD11b$^+$CD33$^+$CD15$^-$CD14$^+$HLA-DR- mMDSCs. (E-H) Representative data for Fig. 2. (E) CD3$^+$, CD4$^+$ and CD8$^+$ T cells before and after PEM treatment. (F) Immunophenotypes of CD4$^+$ T cells. (G) Immunophenotypes of CD8$^+$ T cells. (H) eTregs and mMDSCs. (I and J) Representative data for Fig. 3C. (I) TD CD8$^+$ T cells. (J) Naïve CD4$^+$ T cells. (K) Representative data for Fig. 5C, mMDSCs. (L) Representative data for Fig. 5F, eTregs. eTreg, effector regulatory T cell (FOXP3^{high}CD45RA-CD4$^+$ T cell); mMDSC, monocytic myeloid-derived suppressor cell (CD11b$^+$CD14$^+$CD15$^-$HLA-DR$^-$CD33$^+$); CM, central memory (CD45RA$^-$CD27$^+$CCR7$^+$); EM, effector memory (CD45RA$^-$CD27$^-$CCR7$^-$); naïve, CD45RA$^+$CD27$^+$CCR7$^+$; TD, terminally differentiated (CD45RA$^+$CD27$^-$CCR7$^-$); PEM, pembrolizumab; MNC, mononuclear cell; Lin-, lineage marker negative; FSC-H, forward scatter-height; SSC-A, side scatter-area; FITC, fluorescein isothiocyanate; PE, phycoerythrin; APC, allophycocyanin; AF, Alexa Fluor; BV, brilliant violet; PB, Pacific Blue; PC5, PE-Cyanine5; PC7, PE-Cyanine7; ECD, phycoerythrin-Texas Red conjugate, also known as electron-coupled dye.

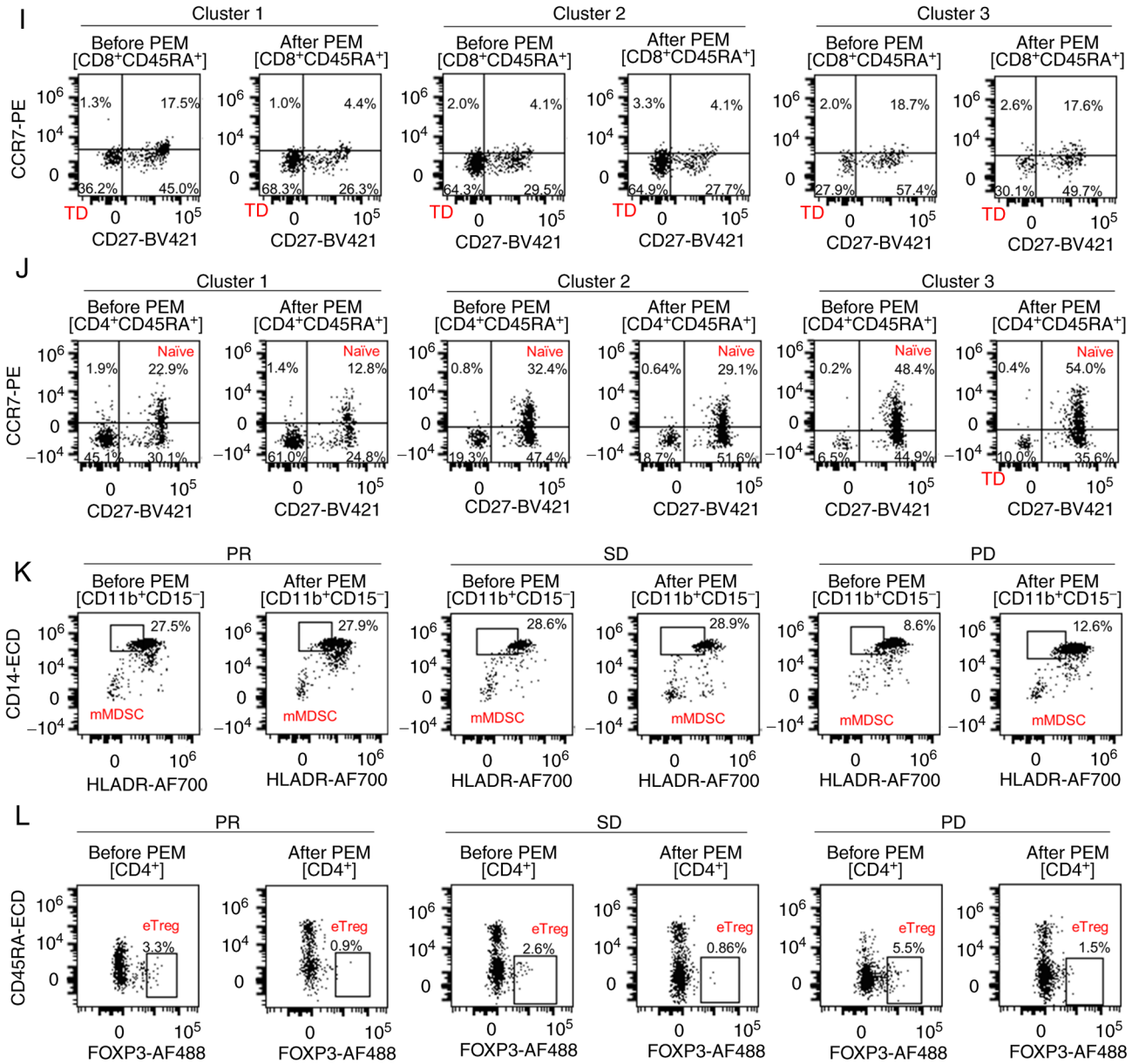

Figure S3. Scatter plot representing the number of chemotherapy courses vs. the changes in cell count of mMDSCs. The linear regression line is shown. The y-axis shows the changes in mMDSC counts before and after pembrolizumab treatment. The x-axis shows the number of chemotherapy courses. mMDSC, monocytic myeloid-derived suppressor cell ($CD11b^{+}CD14^{+}CD15^{-}HLA\text{-}DR^{-}CD33^{+}$).

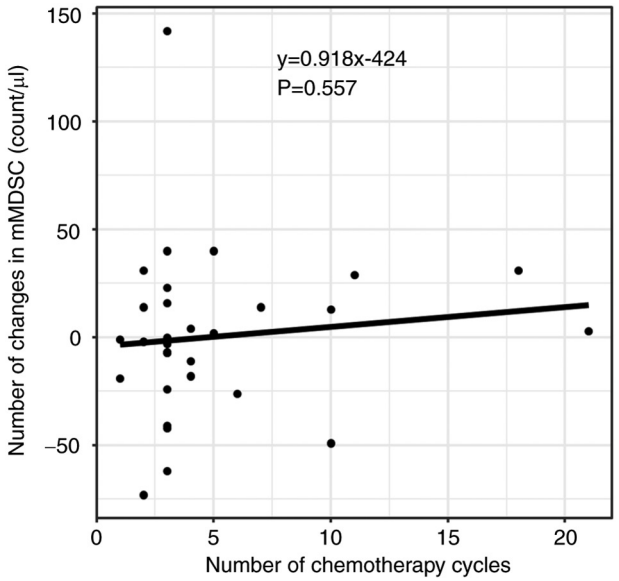

Figure S4. Overall survival compared between two groups according to the high and low ssGSEA score for MDSCs and TAMs. IMvigor210 (NCT02108652) data, from a phase II clinical trial of atezolizumab, were analyzed. Overall survival was compared between patients with high and low ssGSEA scores. (A) Angelova's ssGSEA MDSC score. (B) Cassette's TAM ssGSEA score. These data include 345 tissue samples from patients with muscle-invasive urothelial carcinoma who failed previous platinum-based chemotherapy or previously untreated patients who were ineligible for platinum-based chemotherapy. The expression data and clinical data were obtained from IMvigor 210 Core Biologies (http://research-pub.gene.com/IMvigor210CoreBiologies). Kaplan-Meier curves were plotted and log rank test was performed. MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophage; Treg, regulatory T cell; ssGSEA, single-sample gene set enrichment analysis.