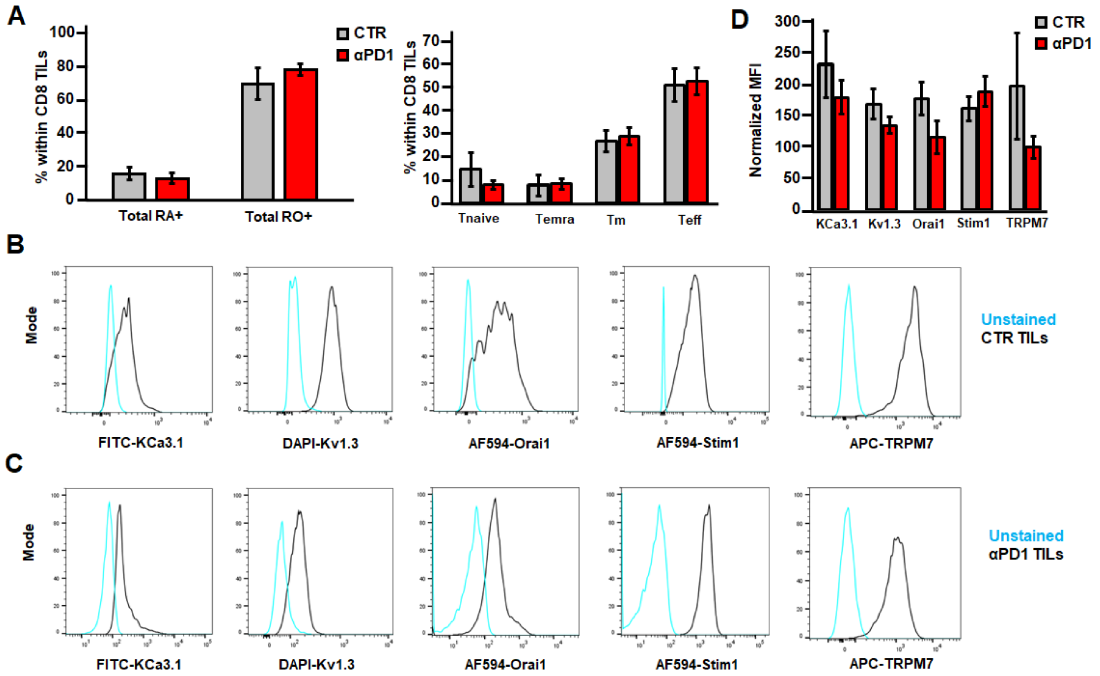

Supplementary Figure 4. T cell phenotype and ion channel expression in TILs. (A) Cells were gated based on the following: live cells, lymphocytes, $CD8^+$ cells and then on memory subtypes (left: CD45RA and CD45RO; right: CD45RA and CD127). Percentage of $CD8^+$ TILs composed of naïve (RA^+) or memory (RO^+) phenotypes in both control (CTR) and pembrolizumab (αPD1) patients (CTR patients n = 9; αPD1 patients n = 17) was determined. Percentage of $CD8^+$ TILs composed of Tnaive, Temra, Tm, and Teff (right) subsets (CTR patients n = 9; αPD1 patients n = 15) was determined. Total RA^+ and total RO^+ were each compared using Mann-Whitney Rank Sum test. Tnaive and Temra subtypes were compared using Mann-Whitney Rank Sum test. Tm and Teff subtypes were each compared using student's t-test. Statistical test determined by data normality and variance. **(B and C)** Representative flow cytometry histograms showing KCa3.1, Kv1.3, Orai1, Stim1, and TRPM7 expression in (B) CTR TILs (black) versus unstained sample (blue) and (C) αPD1 TILs (black) versus unstained sample (blue). Cells were gated based on the following: live cells, lymphocytes, $CD8^+$ cells and then on ion channel expression. **(D)** Ion channel expression are shown in CTR and αPD1 TILs where mean fluorescence intensities (MFIs) of samples were normalized to MFIs of healthy donor sample. KCa3.1 (CTR patients n = 9; αPD1 patients n = 13), Kv1.3 (CTR patients n = 9; αPD1 patients n= 13), Orai1 (CTR patients n = 9; αPD1 patients n = 13), Stim1 (CTR patients n = 7; αPD1 patients n = 9), TRPM7 (CTR patients n = 7; αPD1 patients n = 9). KCa3.1, Kv1.3, Orai1, and TRPM7 results were each compared via Mann-Whitney Rank Sum test. Stim1 results were compared using student's t-test. Statistical test determined by data normality and variance. **(A and D)** All data are represented as mean ± standard error of the mean.