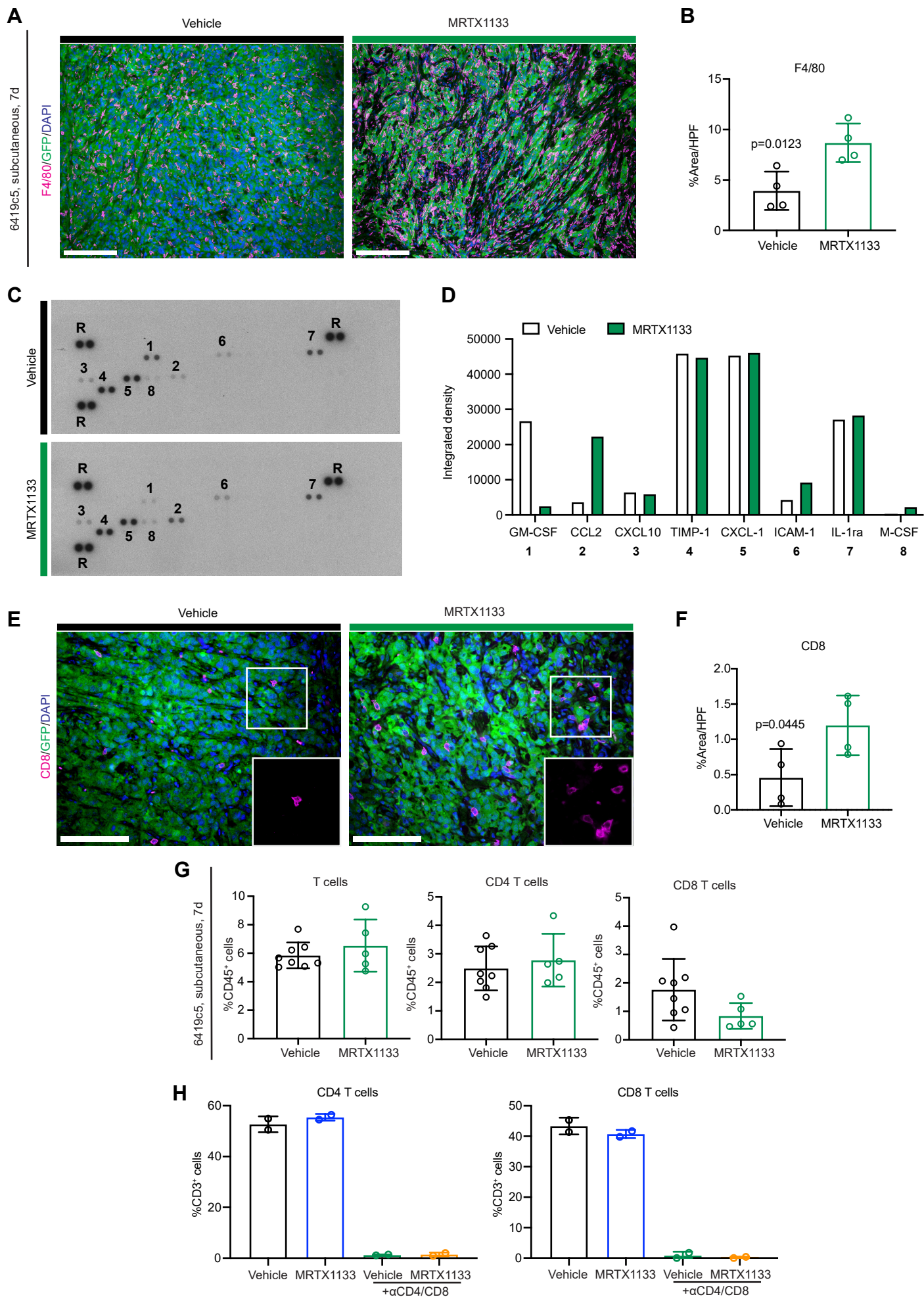


Figure S6

Supplementary Figure 6. KRAS^{G12D} inhibition alters the tumor immune microenvironment.

(A) Representative co-immunofluorescent images of F4/80, GFP, and DAPI in vehicle and MRTX1133 treated tumors (6419c5, subcutaneous, 7d). Scale bars, 100 μ m. Objective, 20x.

(B) Quantitation of macrophages (F4/80⁺) as percent area per high power field (HPF) in vehicle (n= 4) and MRTX1133 treated tumors (n=4). At least 3 fields of view were averaged per tumor. p-values were determined by Student's unpaired t-test. Error bars indicate SD.

(C) Representative proteome profiler cytokine array of conditioned media from 6419c5 tumor cells treated with vehicle (top) or MRTX1133 (bottom) for 48h. R denotes reference spots. Detectable cytokines are numbered 1-8. 1, GM-CSF; 2, CCL2; 3, CXCL10; 4, TIMP-1; 5, CXCL1; 6, ICAM-1; 7, IL-1ra; 8, M-CSF.

(D) Quantitation of cytokine arrays in (C). Integrated density was measured using the protein array analyzer macro in FIJI. Results representative of two independent experiments.

(E) Representative co-immunofluorescent images of CD8, GFP, and DAPI in vehicle and MRTX1133 treated tumors (6419c5, subcutaneous, 60h). Inset represents higher magnification of CD8⁺ T cells in boxed area. Scale bars, 100 μ m. Objective, 40x.

(F) Quantitation of CD8 T cells (CD8⁺) as percent area per high power field (HPF) in vehicle (n= 4) and MRTX1133 treated tumors (n=4). At least 3 fields of view were averaged per tumor. p-values were determined by Student's unpaired t-test. Error bars indicate SD.

(G) Flow cytometry of indicated T cell subsets from 6419c5 (subcutaneous) tumors after 7d of treatment with vehicle (n=8) or MRTX1133 (n=5). p-values were determined by Student's unpaired t-test. Error bars indicate SD.

(H) Flow cytometry of T cell subsets (as %CD3) from the blood of tumor-bearing (2838c3) animals after 8 days of treatment of vehicle (n=2), MRTX1133 (n=2), vehicle + α CD4/CD8 (n=2), and MRTX1133 + α CD4/CD8 (n=2).