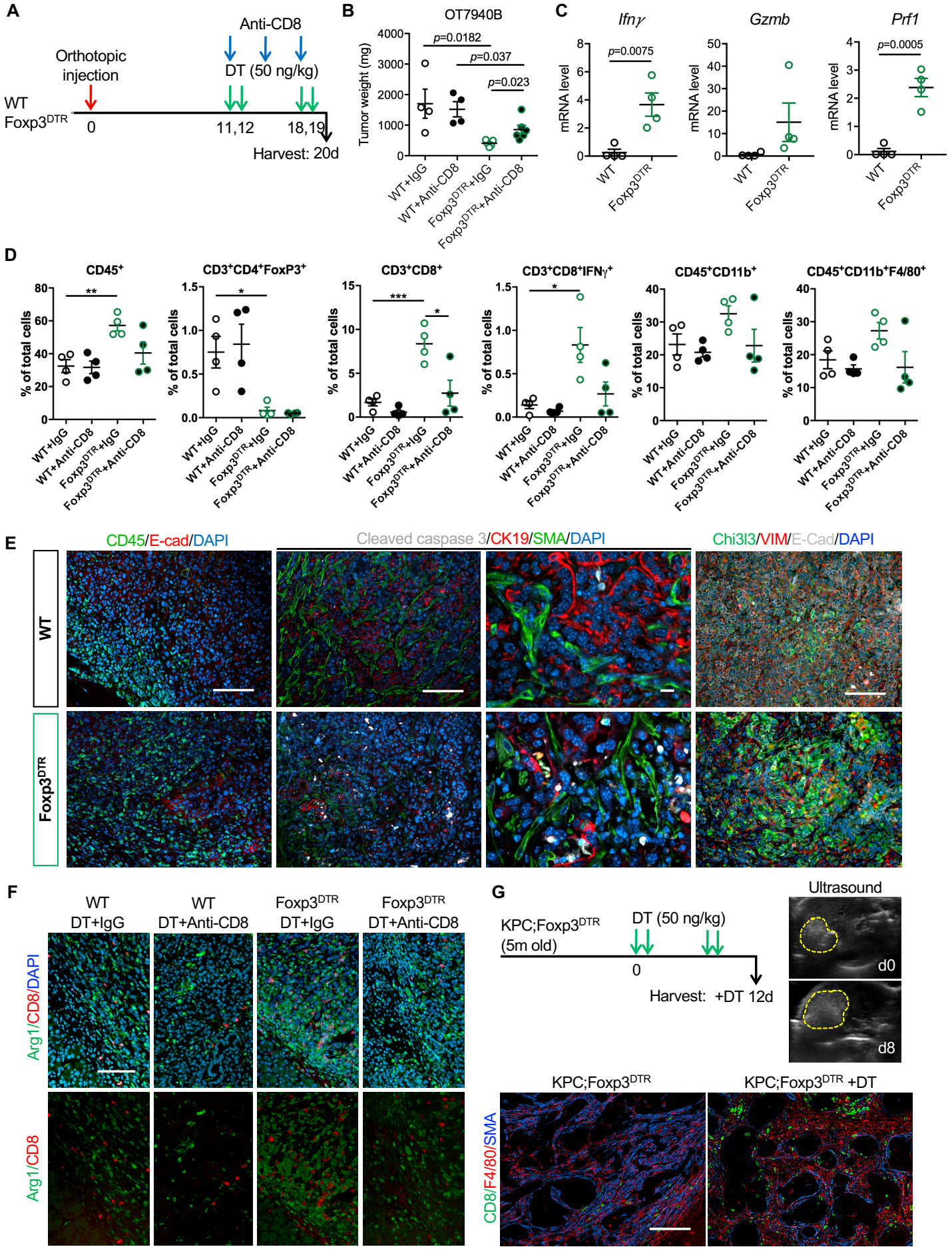

Supplemental Figure 4

Supplemental Figure 4. Treg depletion in pancreatic cancer results in increased CD8 T cell and immunosuppressive factors. (A) Experimental design, n=4 mice/cohort. **(B)** Tumor weights of orthotopic PDA harvested from WT and Foxp3^{DTR} mice received DT or combination of DT and anti-CD8. Data represent mean ± SEM, n=4. The statistical difference was determined by two-tailed t-tests. **(C)** qRT-PCR for *Ifnγ, Gzmb and Prf1* expression in WT and Foxp3^{DTR} orthotopic tumors received DT. Data represent mean ± SEM, n=4. The statistical difference was determined by two-tailed t-tests. **(D)** CD45^{+} leukocytes, CD3^{+}CD4^{+}FoxP3^{+} Tregs, CD3^{+}CD8^{+} T cells, CD3^{+}CD8^{+}IFNγ^{+} T cells, CD11b^{+} myeloid cells and CD11b^{+}F4/80^{+} macrophages were measured by flow cytometry. Data represent mean ± SEM. The statistical difference was determined by two-tailed t-tests. **(E)** Co-immunofluorescent staining for CD45 (green), E-cad (red) and DAPI (blue), or for SMA (green), CK19 (red), Cleaved caspase 3 (grey) and DAPI (blue), or for Chi3l3 (green), VIM (red), E-cad (grey) and DAPI (blue). (**F**) Co-immunofluorescent staining for Arg1 (green), CD8 (red) and DAPI (blue) in orthotopic PDA tumors. Scale bar 100 µm. **(G)** Experimental scheme for depletion of Tregs in a KPC; Foxp3^{DTR} **(**ptf1aCre; LSL-Kras; P53$^{R172H/+;}$ Foxp3^{DTR}) mouse. *Top right*: Ultrasound images showing the pancreatic tumor (highlighted by yellow dash lines) before and 8 days after DT treatment show continued tumor growth. *Bottom*: Co-immunofluorescent staining for CD8 (green), F4/80 (red) and SMA (blue) in KPC; Foxp3^{DTR} pancreatic tumor. Scale bar 100 µm.