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Supplemental Information

Pulsatile MEK Inhibition Improves Anti-tumor Immunity and T Cell Function in Murine Kras Mutant Lung Cancer

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Figure S1 (Related to Figure 1)

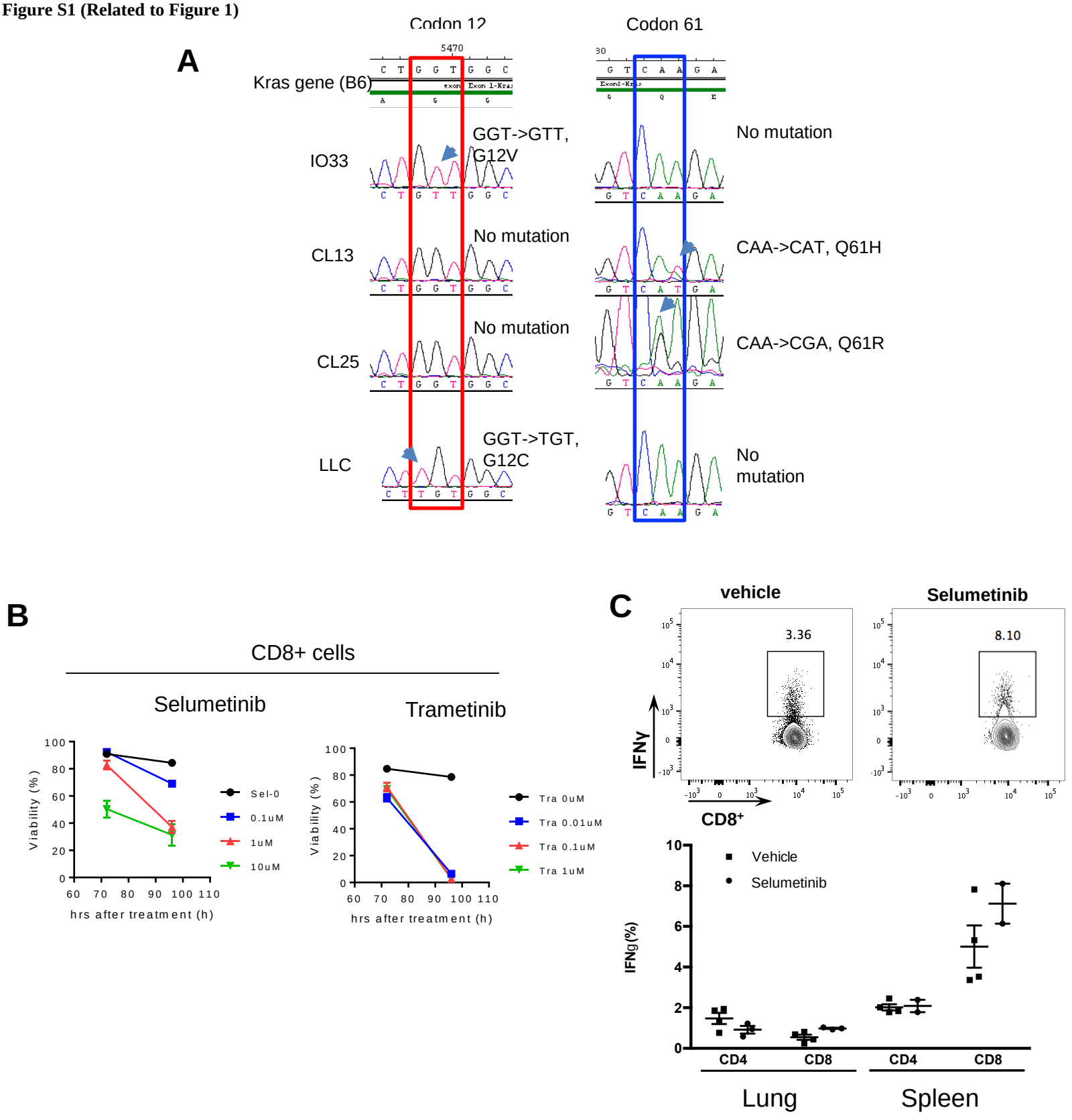

Figure S1. Related to Figure 1. **A.** Kras mutations from diverse murine lung cancer cell lines. Sequencing histogram of codon 12 or codon 61 of Kras gene from IO33, CL13 CL25, and LLC lung cancer cell lines. Red and blue boxes indicate codon 12 and codon 61. Blue arrows indicate changes in a sequence in individual cell lines. **B.** Viability measured by flow cytometry using fixable viability dye after different concentrations of selumetinib (left) or trametinib (right) on splenocytes that were activated with anti-CD3 and anti-CD28 antibodies. **C.** IFNγ expression by flow cytometry of T cells from lung and spleen of tumor-bearing mice (four mice from control group and three mice from treatment group).

Figure S2 (Related to Figure 2)

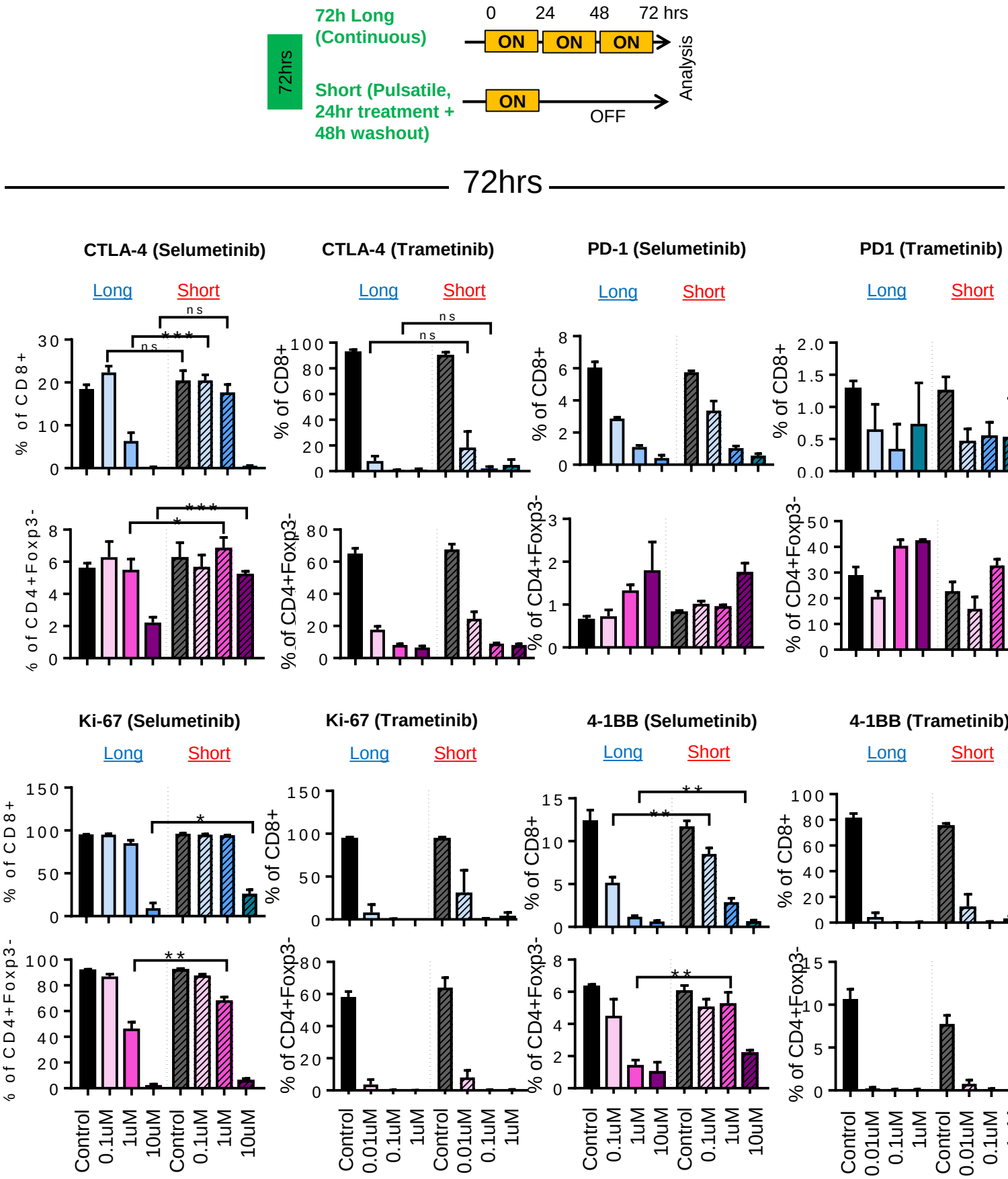

Figure S2. Pulsatile schedule of MEKi treatment altered T cell activation status *in vitro*, Related to Figure 2. CTLA-4, PD1, Ki-67, and 4-1BB expression in CD8+ cells and CD4+Foxp3- cells from spleen of HKP1 lung tumor-bearing mice by flow cytometry after selumetinib (left) and trametinib (right) treatment for 72 hrs. Welch's test, * <0.05, **<0.01, ***<0.001

Figure S3 (Related to Figure 3)

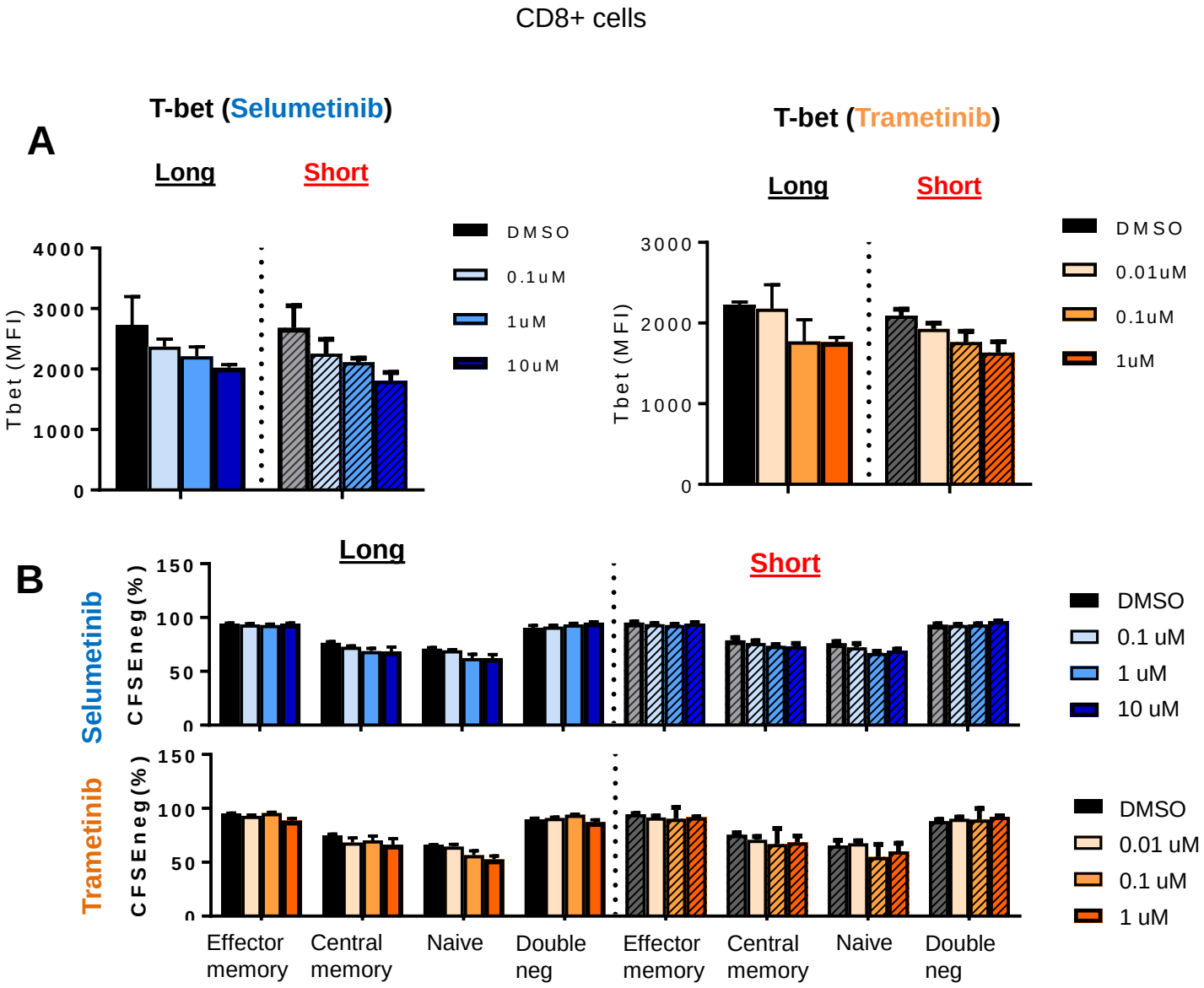

Figure S3. Flow cytometry analysis of primed Pmel-1 CD8+ cells after continuous or pulsatile treatment of MEK inhibitors during priming, Related to Figure 3. **A.** Expression of Tbet from Pmel-1 CD8+ cells after long or short treatment of MEKis by flow cytometry. **B.** Frequency of CFSE negative cells from subsets of CD44 and CD62L combination.

Figure S4 (Related to Figure 4)

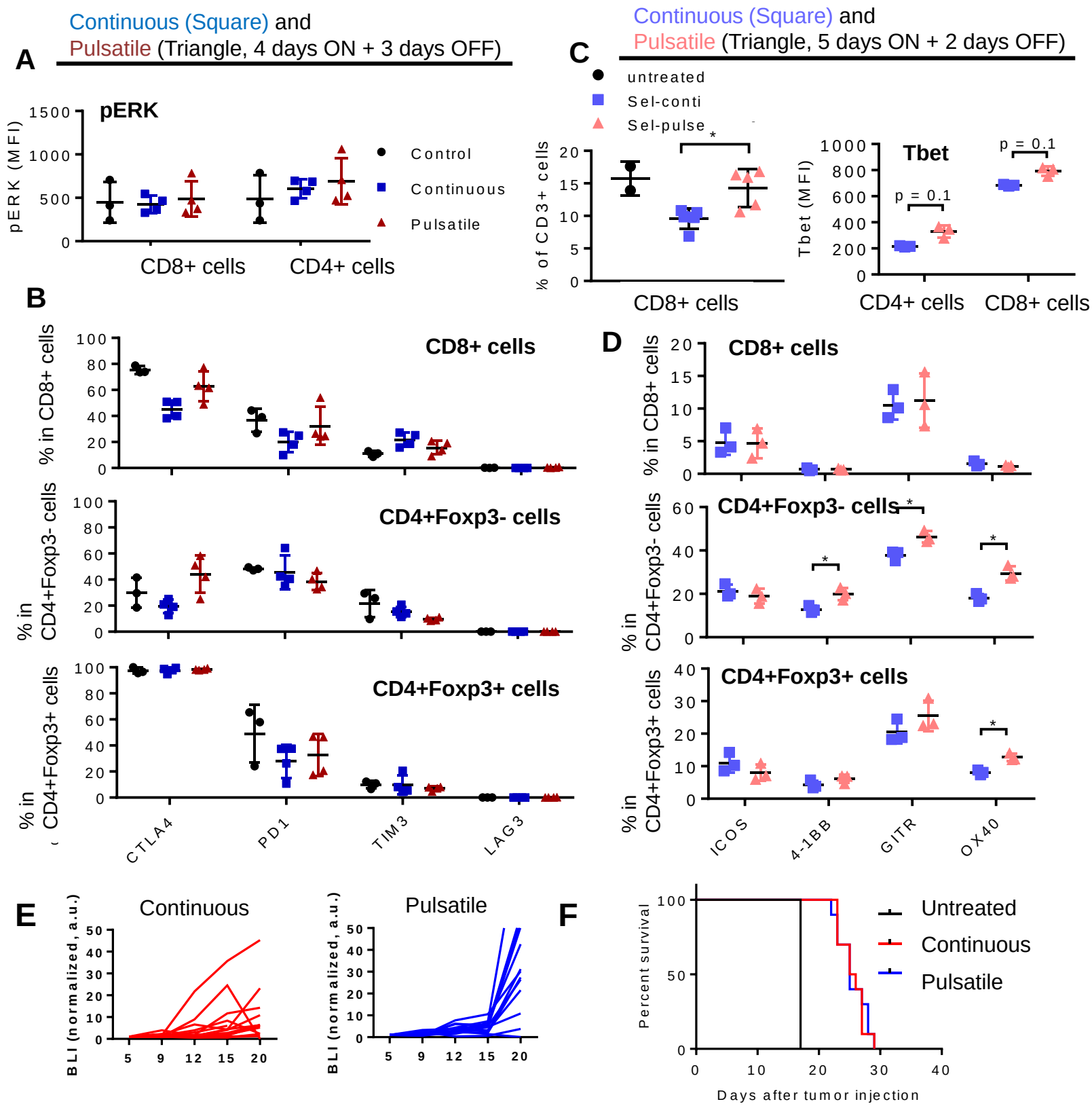

Figure S4. T cells show differential phenotypes with continuous and pulsatile selumetinib treatment, Related to Figure 4. HKP1 lung tumor-bearing mice were treated for 2 weeks either continuously with 25mg/kg of selumetinib twice a day (continuous group) or with pulsatile dosing with 2 cycles of treatment. Pulsatile treatment was done with 300 mg/kg twice a day, 4 days ON and 3 days OFF (Figures A, B) or with 25 mg/kg twice a day, 5 days ON and 2 days OFF (Figures C-F). The control group was treated with vehicle continuously. All mice were sacrificed, and lungs were collected and analyzed by flow cytometry after 2 weeks of treatment. **A.** pERK expression in CD8+ cells and CD4+ cells. **B.** Co-inhibitory markers expression of T cells. **C.** T cell infiltration in tumor lung (left) and Tbet and Eomes expression from T cells in tumor lungs (right). **D.** Co-stimulatory markers expression of T cells in HKP1 tumor lungs. Mann Whitney, * <0.05. **E.** Tumor growth measured by bioluminescence from 5+2 days treatment experiment. **F.** Survival of tumor-bearing mice from 5+2 days treatment.

Figure S5 (Related to Figure 5)

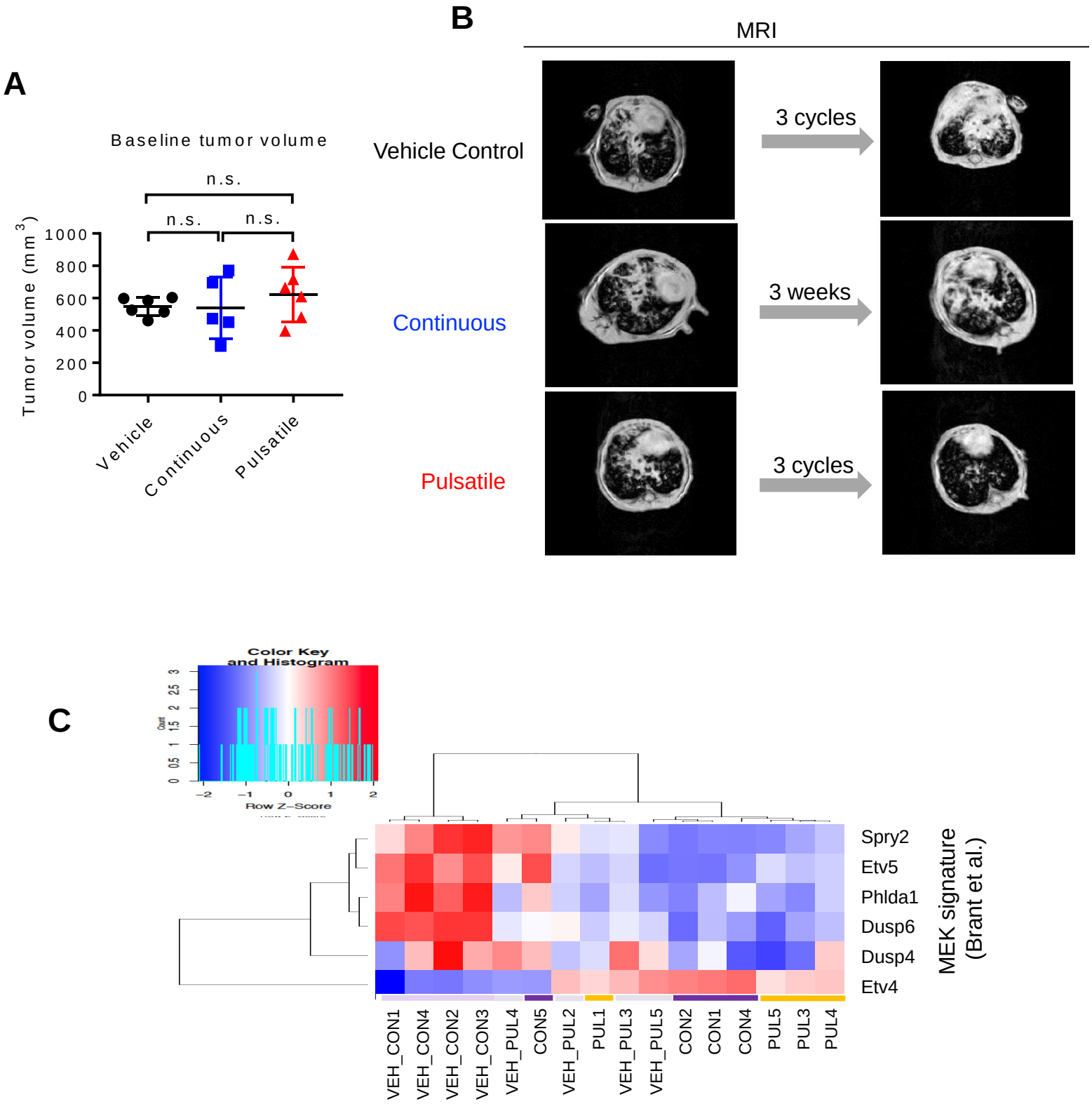

Figure S5. *KRAS*^{G12C} mice treated with pulsatile or continuous selumetinib, Related to Figure 5**. A.** The starting tumor volume of *KRAS*^{G12C} mice with individual treatments at baseline. **B.** Representative images of MRI of ^{KRASG12C} mice before and after treatment with pulsatile selumetinib, continuous selumetinib or vehicle control. **C.** MEK signature from transcriptome analysis of lung tumors. VEH_CON (vehicle for continuous group), CON (continuous group), VEH_PUL (vehicle for pulsatile group), PUL (pulsatile group). Each group has 4 - 5 mice for this analysis.

Figures S6 (Related to Figure 6)

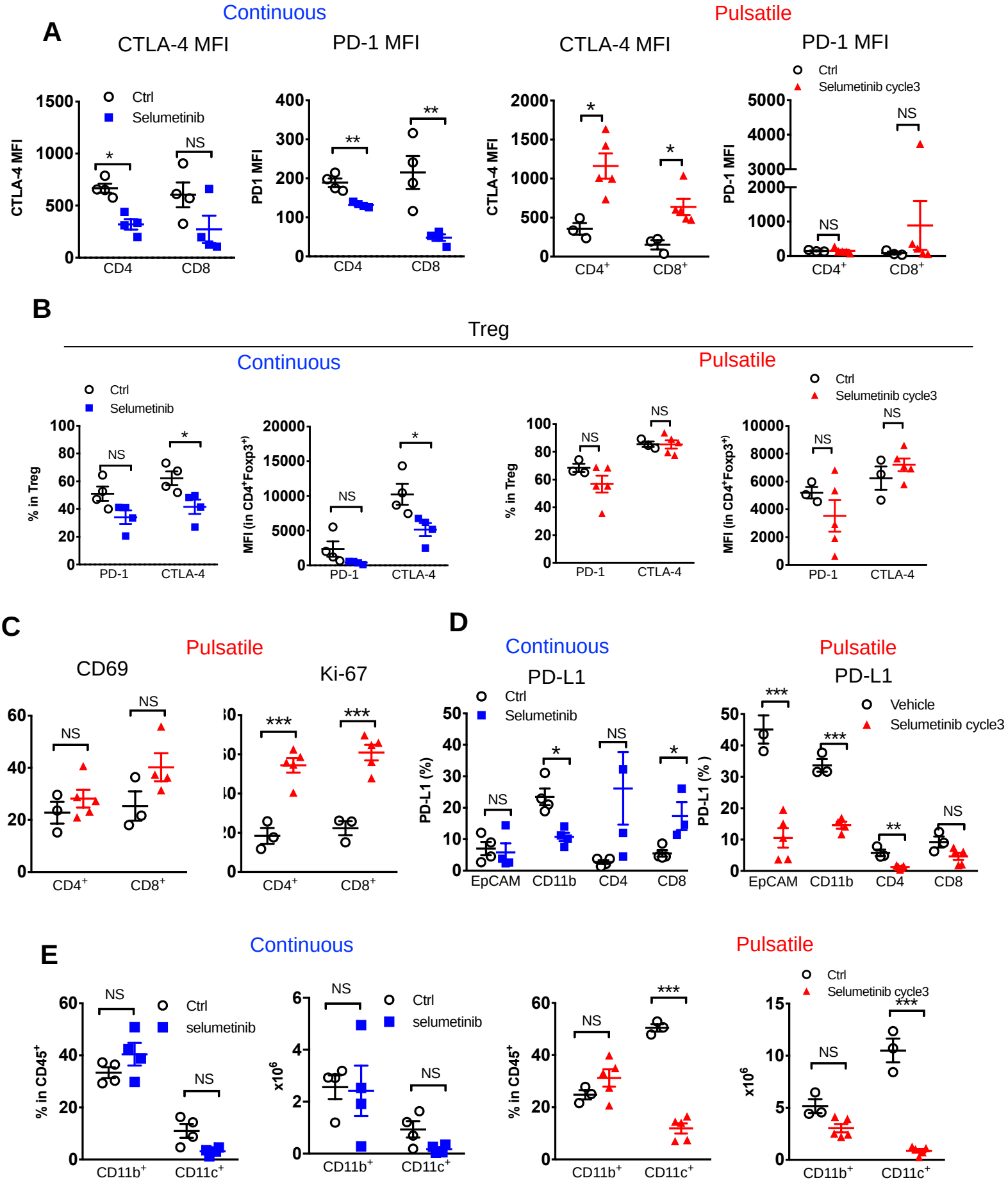

Figure S6. Flow cytometry analysis of selumetinib treated *KRAS*^{G12C} GEMM tumor from Figure 5 experiment, Related to Figure 6. **A.** MFI of CTLA-4 and PD-1 from CD4+ cells and CD8+ cells. **B.** CTLA-4 and PD-1 of Treg cells gated as CD4+Foxp3+ cells. **C.** CD69 and Ki-67 from pulsatile treated mice. **D.** PD-L1 from continuous and pulsatile treatment **E.** Myeloid cells infiltration from continuous and pulsatile treatment. ** < 0.01; *** < 0.001

Figures S7 (Related to Figure 7)

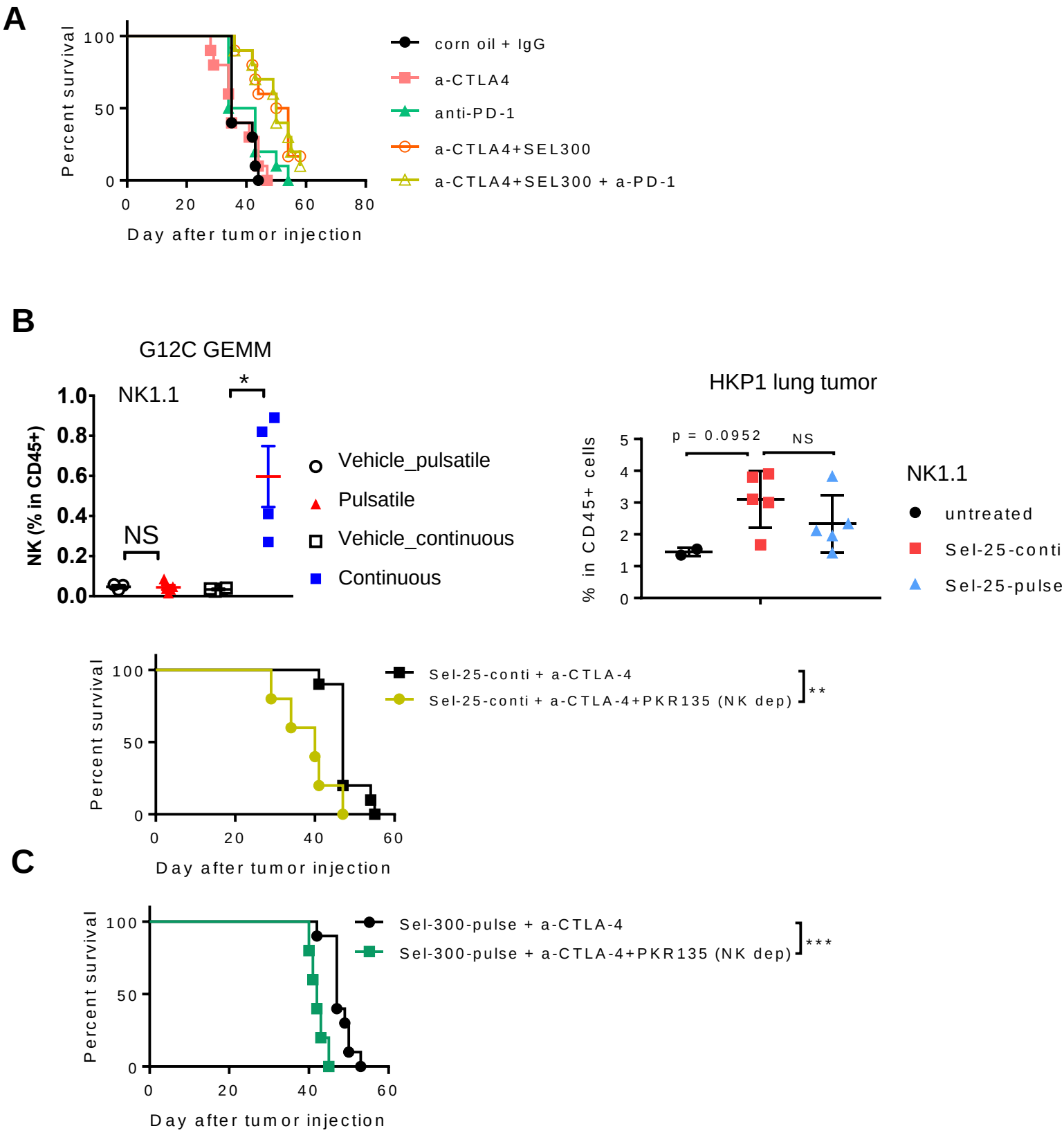

Figure S7. Related to Figure 7. **A.** Survival of anti-PD-1 mono/combination therapy in transplantable LLC model. The experiments were performed twice and representative result was presented. **B.** NK cell infiltration in lung tumors from GEMM and HKP1 transplantable model. **C.** Survival of selumetinib + anti-CTLA-4 treatment with/without NK cells depletion in transplantable LLC model, 4 weeks of treatment. Pulsatile treatment: 4 days ON + 3 days OFF (n = 5). Flow cytometry analysis and survival analysis were performed once. *<0.05; **<0.01; ***<0.001.