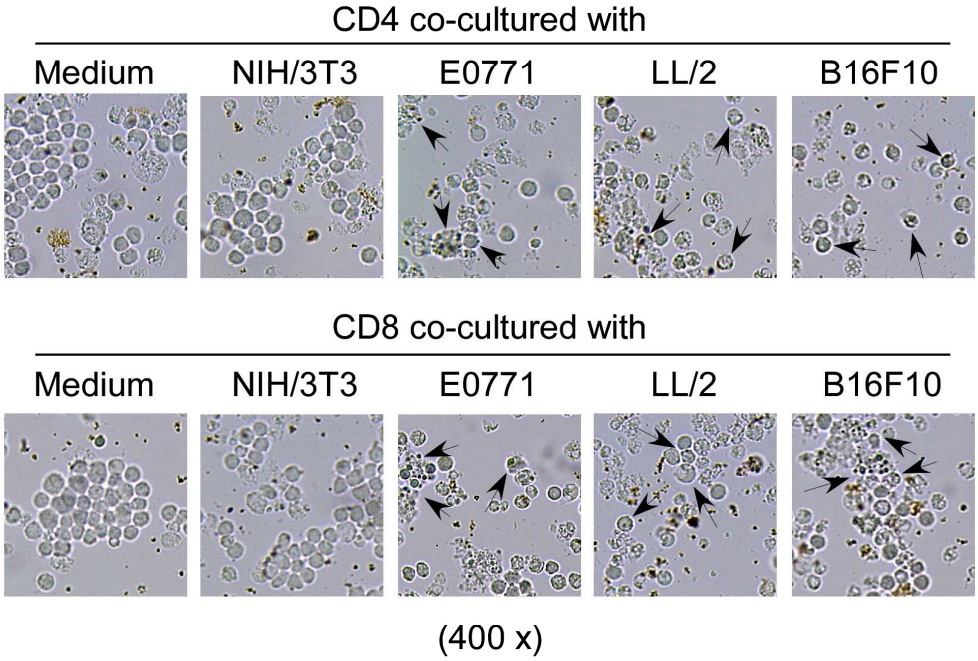
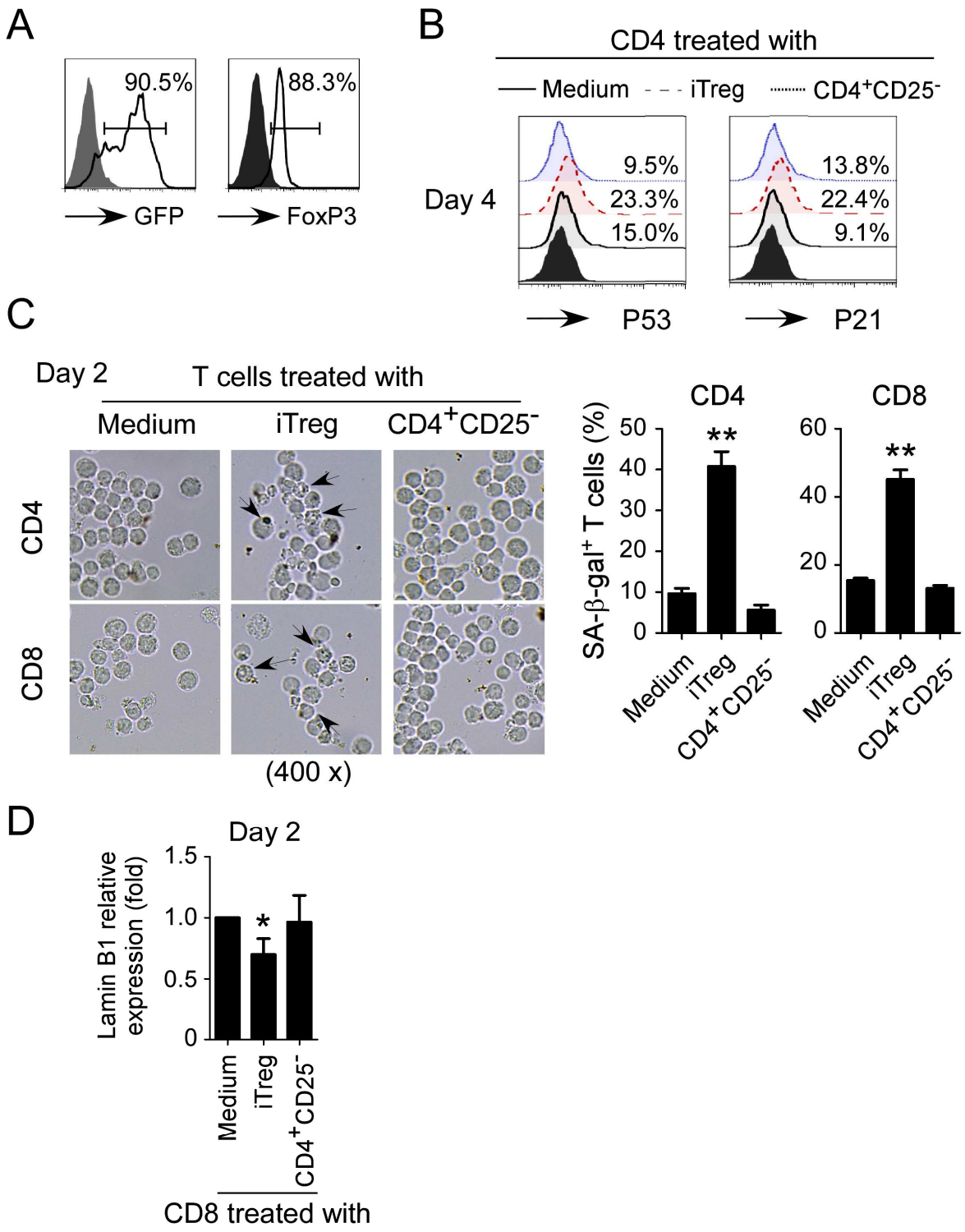
Supplemental Figure 1



Supplemental Figure 1. Mouse tumor cells induce T cell senescence.

Co-culture with different types of mouse tumor cells significantly increased SA-β-gal$^+$ T cell populations in co-cultured T cells. However, T cells co-cultured with the embryonic fibroblast cell line NIH/3T3 had little or no SA-β-gal expression. Cell treatment and culture procedure were the same as in Figure 1B. SA-β-gal$^+$ cells were indicated as dark blue granules in the cytoplasm.

Supplemental Figure 2



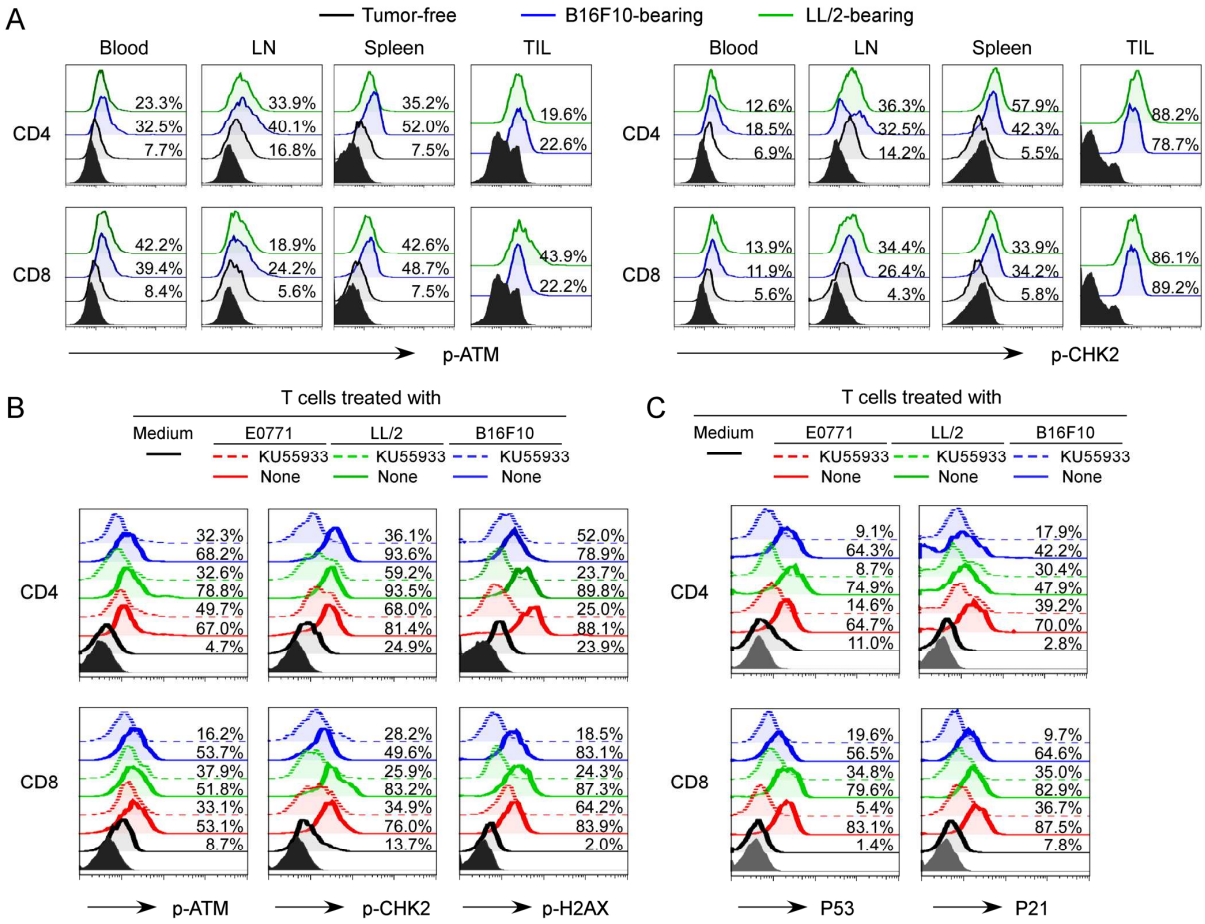
Supplemental Figure 2. Mouse iTreg cells induce T cell senescence.

(A) Purified $CD4^+$ T cells from the spleens of $Foxp3^{eGFP}$ reporter mice were differentiated into iTreg cells in the presence of rhTGF-β and rhIL-2 for 5 days. GFP and FoxP3 expression in the induced T cells were further confirmed by flow cytometry analyses.

(B) iTregs promoted the expression of cell cycle regulatory molecules P53 and P21 in mouse $CD4^+$ T cells. Cell treatments and ratios were identical to the respective experiments in Figure 2A and cultured for 4 days. P53 and P21 expression was determined by flow cytometry analysis.

(C) and **(D)** iTreg cell treatment induced the increase of SA-β-gal^+ T cell populations and loss of Lamin B1 expression in mouse responder T cells. T cells were co-cultured with iTreg cells at a ratio of 4:1 in the presence of plate-bounded anti-CD3/CD28 antibodies for 2 days. The treated T cells were stained for SA-β-gal (in C). The SA-β-gal^+ T cells were identified with dark blue granules indicated by the arrows. mRNA expression levels of Lamin B1 were determined by real-time PCR analyses (in D). The expression level was normalized to HPRT expression and adjusted to the levels in the T cell alone group. Data are mean ± SD from 3 independent experiments. *$p<0.05$ and **$p<0.01$, compared with the respective medium only group. Paired Student's *t*-test was performed.

Supplemental Figure 3



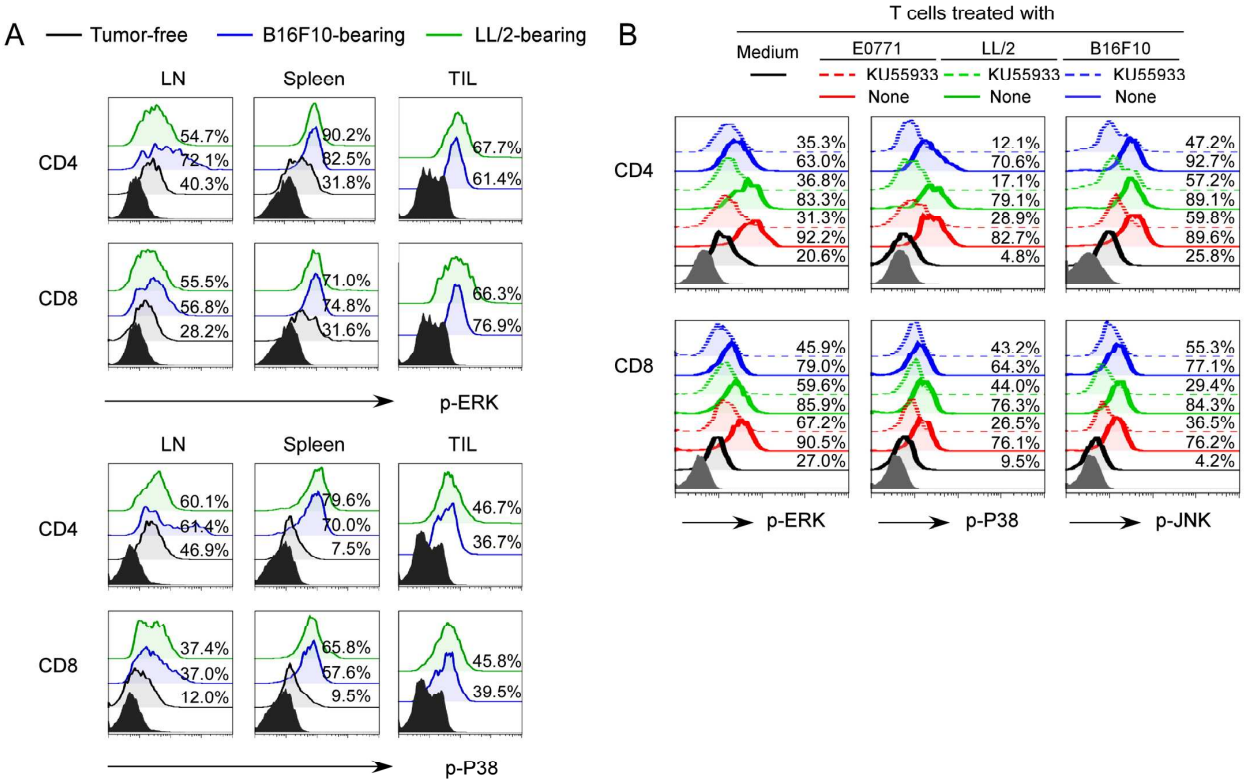
Supplemental Figure 3. DNA damage response involves T cell senescence induced by tumor cells.

(A) T cells derived from different organs and tumors in E0771, LL/2, and B16F10 tumor-bearing mice had increased phosphorylation of ATM and CHK2. Blood, LNs, spleens, and tumor tissues were harvested from the tumor-bearing mice when primary tumors reached 10-15 mm in diameters. T cells purified from tumor-free littermates served as controls. The p-ATM and p-CHK2 expressions in T cells were analyzed by the flow cytometry.

(B) Treatment with KU55933 dramatically reversed the increased phosphorylation of ATM, H2AX, and CHK2 in T cells induced by tumor cells. Preactivated $CD4^+$ and $CD8^+$ T cells were treated with ATM inhibitor KU55933 (10 μM) for 24 hours and then co-cultured with indicated tumor cells at a ratio of 1:1 for 1 day. The treated T cells were separated and cultured for additional 5 days. The p-ATM, p-H2AX, and p-CHK2 expression levels were determined by flow cytometry analysis.

(C) Treatment with KU55933 significantly prevented the increased levels of P53 and P21 in T cells induced by tumor cells. Cell treatments and ratios were identical to the respective experiments in (B). P53 and P21 expression levels were determined by flow cytometry analysis.

Supplemental Figure 4

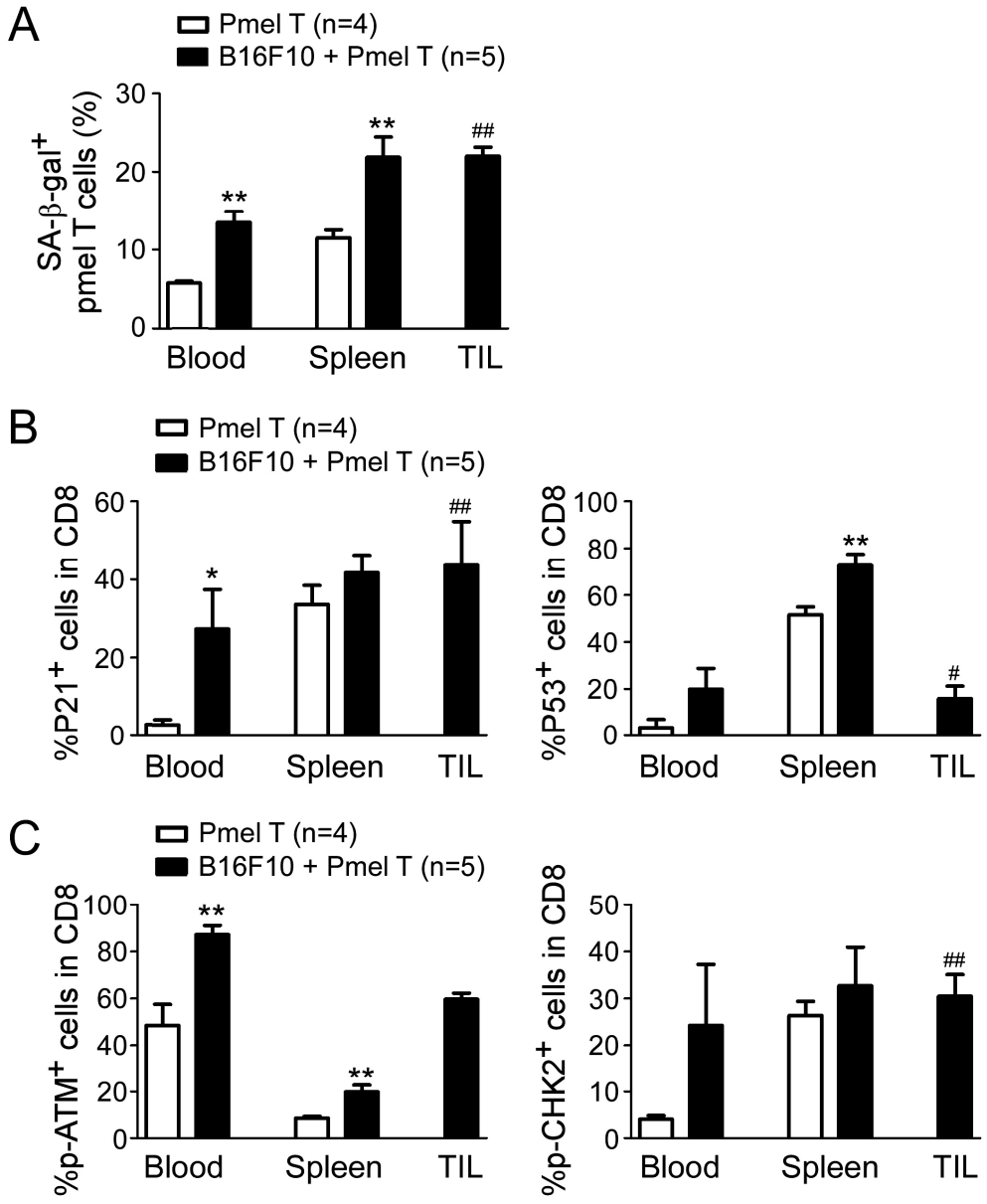


Supplemental Figure 4. MAPK signaling involves T cell senescence induced by tumor cells.

(A) T cells derived from different organs and tumors in E0771, LL/2, and B16F10 tumor-bearing mice had increased phosphorylation levels of ERK and P38. LNs, spleens and tumor tissues were harvested from the tumor-bearing mice when primary tumors reached 10-15 mm in diameter. T cells purified from tumor-free littermates served as controls. The p-ERK and p-P38 expression levels in treated T cells were analyzed by the flow cytometry.

(B) Treatment with KU55933 dramatically reversed the increased phosphorylation levels of ERK, P38, and JNK in T cells induced by tumor cells. Preactivated $CD4^+$ and $CD8^+$ T cells were treated with ATM inhibitor KU55933 (10 μM) for 24 hours and then co-cultured with different tumor cells at a ratio of 1:1 for 1 day. The treated T cells were separated and cultured for an additional 5 days. The p-ERK, p-P38, and p-JNK expression levels were determined by flow cytometry analysis.

Supplemental Figure 5

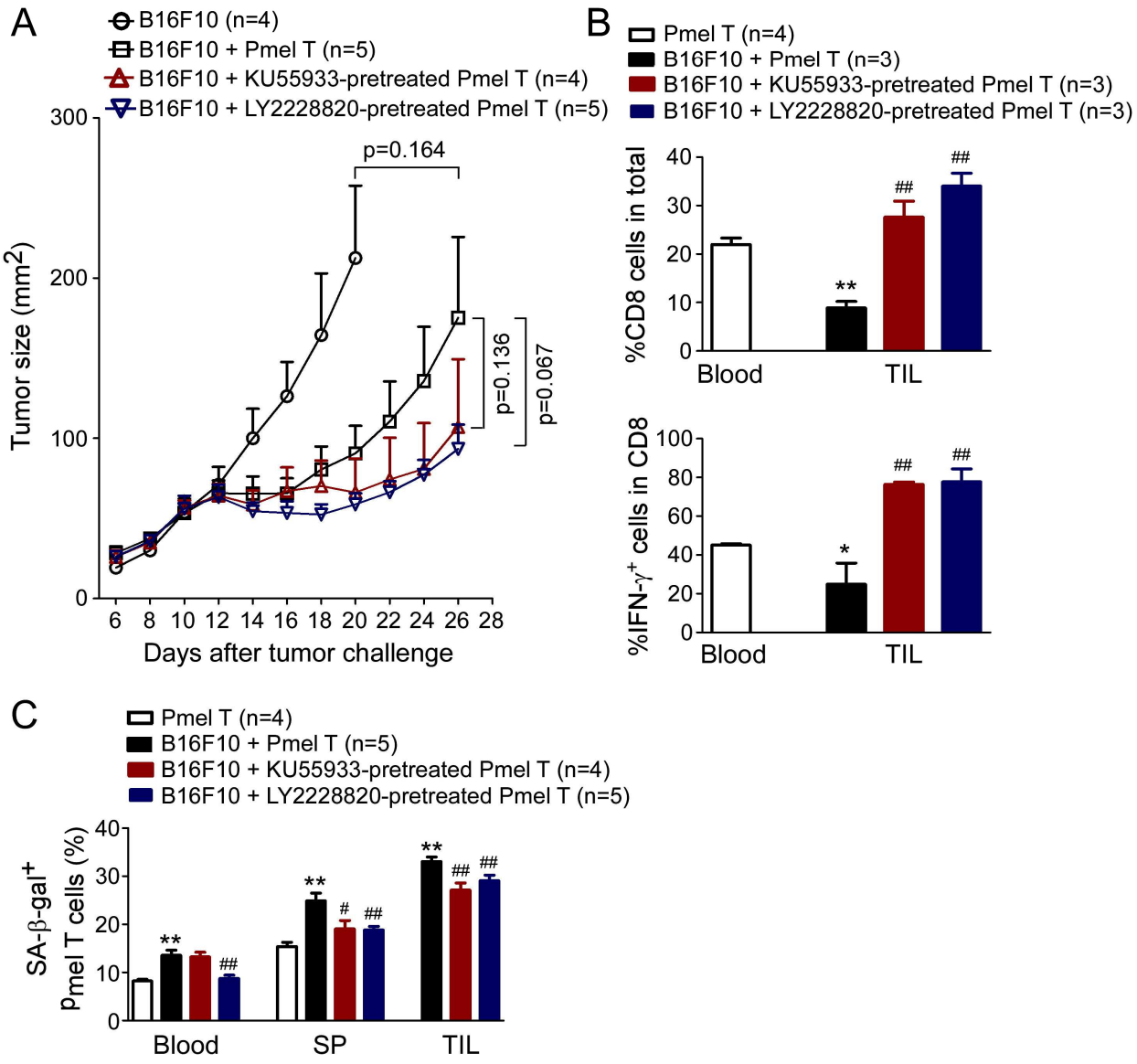


Supplemental Figure 5. Mouse B16 melanoma tumor cells promote senescence development in tumor-specific T cells in vivo.

(A) Accumulated senescent Pmel-1 T cells existed in different organs and tumor tissues in B16F10 tumor-bearing $Rag1^{-/-}$-immunodeficient mice. Mouse melanoma B16F10 cells (2×10^5/mouse) were subcutaneously injected into the $Rag1^{-/-}$-immunodeficient mice. The anti-CD3/CD28-activated Pmel-1 T cells (2×10^6/mouse) were adoptively transferred through i.v. injection into B16F10-bearing $Rag1^{-/-}$ mice at day 6 post tumor inoculation. Blood, spleens and tumor tissues were harvested when tumors reached 10-15 mm in diameter and the transferred Pmel-1 T cells were isolated and stained for SA-β-gal. Data shown are mean ± SD from 4-5 mice each group. **p<0.01, compared with the respective Pmel-1 T cells from tumor-free mice. $^{\#\#}$p<0.01, compared with Pmel-1 T cells purified from blood in tumor-free mice.

(B) and **(C)** T cells purified from different organs and tumors in B16F10 tumor-bearing $Rag1^{-/-}$ mice had an increased expression of P21 and P53 (in B), as well as activated phosphorylation of ATM and CHK2 (in C). Cell transfer procedures and purification were as described in (A). Expression of P21, P53, p-ATM and p-CHK2 in purified T cells was analyzed by the flow cytometry. Data shown are mean ± SD from 4-5 mice in each group. *p<0.05 and **p<0.01, compared with the respective T cells in tumor-free mice. $^{\#}$p<0.05 and $^{\#\#}$p<0.01, compared with the T cells purified from blood in tumor-free mice. Unpaired Student's *t*-test was performed in A, B and C.

Supplemental Figure 6



Supplemental Figure 6. Pretreatment with ATM or P38 inhibitors in Pmel-1 T cells prevents T cell senescence and enhanced anti-tumor immunity against melanoma.

(A) Pmel-1 T cells pretreated with KU55933 or LY 2228820 enhanced anti-tumor immunity against B16F10 melanoma. Mouse B1610 tumor cells (2×10^5/mouse) were subcutaneously injected into C57BL/6 mice. The activated Pmel-1 T cells (2×10^6) were pretreated with/without KU55933 (10 μM) or LY2228820 (1 μM) overnight and then adoptively transferred through i.v. injection into B16F10-bearing mice at day 6 post tumor inoculation. Tumor volumes were measured and presented as mean ± SD (n=4-5 mice/group).

(B) KU55933 or LY2228820 pretreatment in T cells significantly increased $CD8^+$ cell fraction and IFN-γ^+ populations in Pmel-1 T cells from tumor tissues in B16F10-bearing mice. Blood and tumors were harvested at day 27 post tumor injection. Lymphocytes were separated from blood and tumor tissues, and T cell subpopulations were analyzed by flow cytometry. Data shown are mean ± SD from different groups (n=3-4 mice/group). *p<0.05 and **p<0.01, compared with the adoptive transfer T cells purified from blood in tumor-free mice. $^{\#\#}$p<0.01, compared with the adoptive transfer T cells in tumor-bearing mice without inhibitor pretreatment group.

(C) Pretreatment with inhibitors prevented senescence induction in transferred Pmel-1 T cells in B16F10-bearing mice. The transferred Pmel-1 T cells in different organs and tumors were isolated and stained for SA-β-gal. Data shown are mean ± SD from 4-5 mice each group. **p<0.01, compared with the adoptive transfer T cells from blood in tumor-free mice. $^{\#}$p<0.05 and $^{\#\#}$p<0.01, compared with the adoptive transfer T cells in tumor-bearing mice without inhibitor pretreatment group.