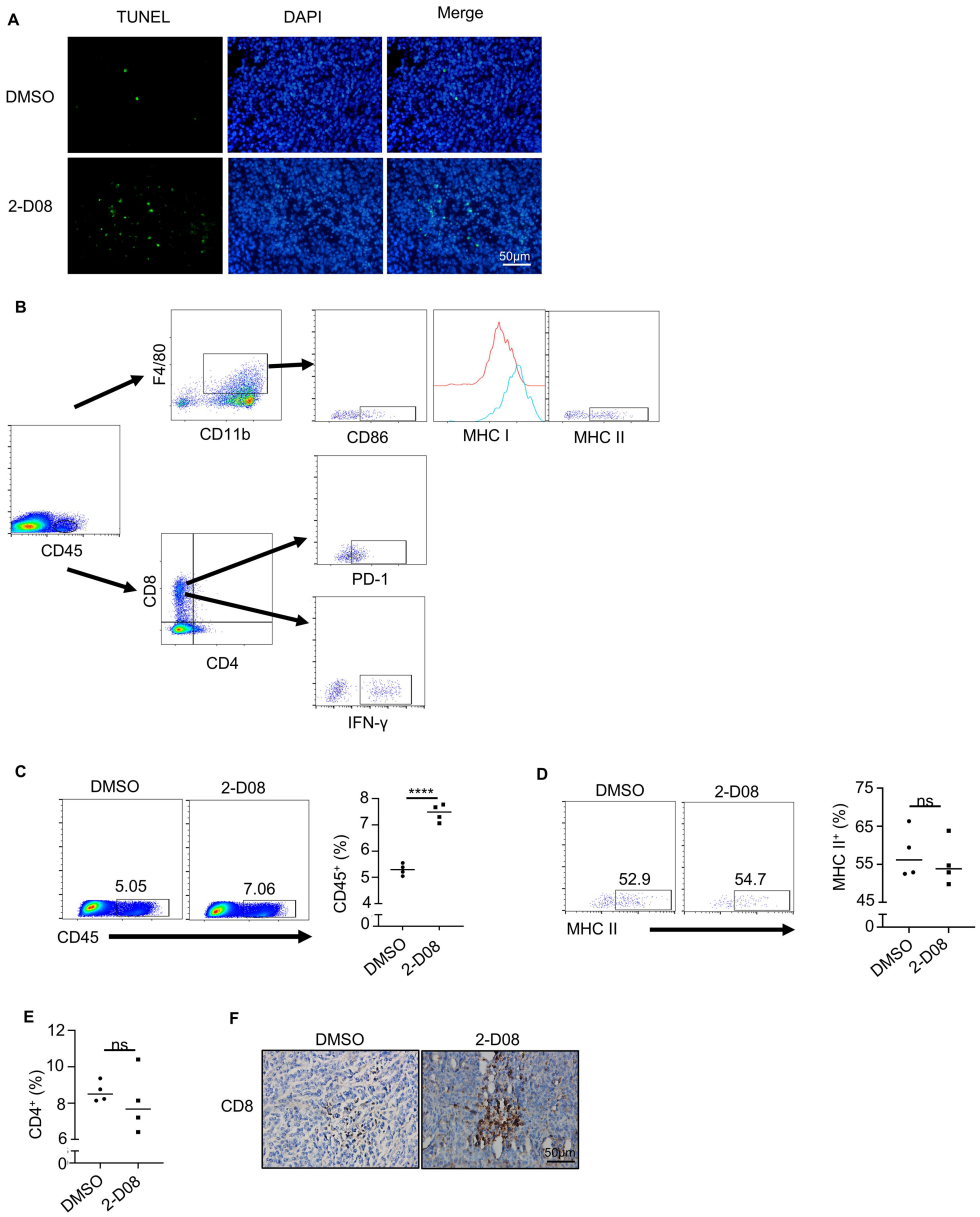
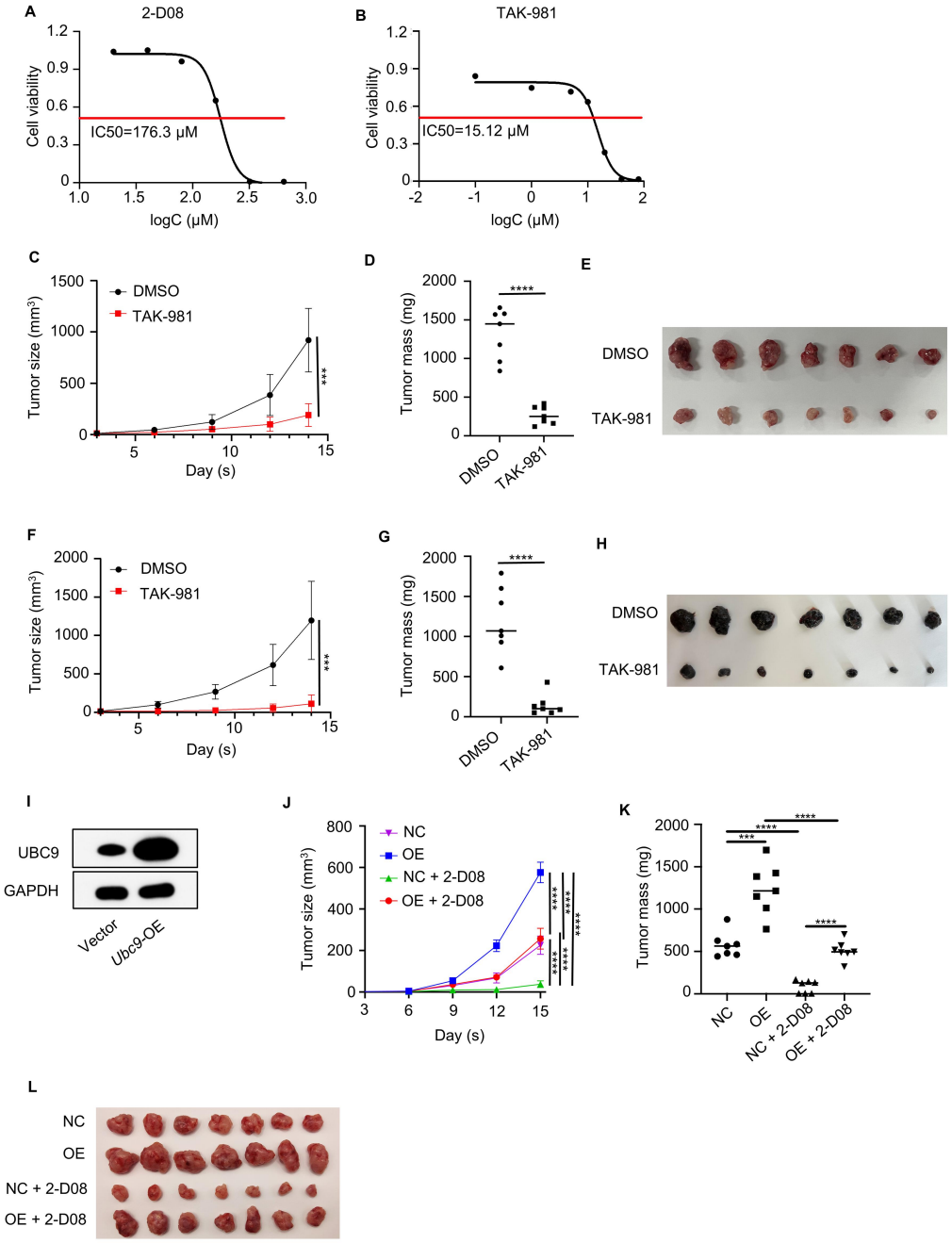
Supplementary Figures:

Supplemental Figure 1.



2-D08 promotes prostate tumor apoptosis and facilitates immune cell infiltration. (**A**) TUNEL staining of tumors in the DMSO and 2-D08-treated mice. (**B**) Gating strategy for infiltrating immune cells in prostate tumors. (**C**, **D** and **F**) $CD45^+$ immune cells (**C**), MHC II^+ macrophages (**D**), and $CD4^+$ T cells (**E**) in tumors compared in the DMSO and 2-D08 treated mice, respectively. (**F**) IHC staining of $CD8^+$ T cells in the two groups. (**A**) and (**F**) represent results from three independent experiments. Data in (**C-E**) represent mean ± SEM and were analyzed by Student's *t*-test (n = 4 / group). ****$P < 0.0001$; ns, not significant.

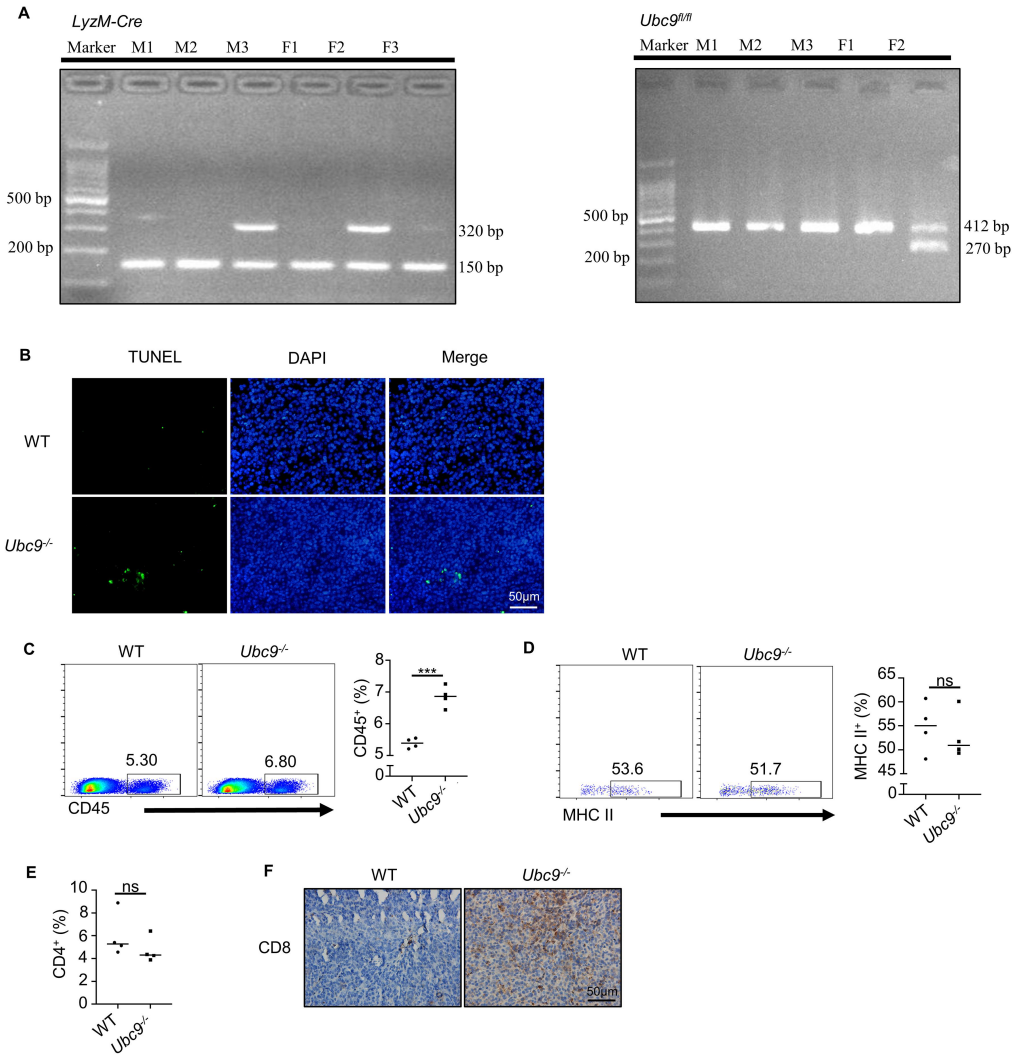
Supplemental Figure 2.



SUMOylation inhibitors repress tumor cell proliferation and progression. (**A** and **B**) The cell viability of prostate cancer cell line RM-1 treated with SUMOylation inhibitors, 2-D08 (**A**) and TAK-981 (**B**) *in vitro*. (**C**, **D** and **E**) Prostate tumor growth curve (**C**) and tumor mass (**D** and **E**) in nude mice at day 14 treated with DMSO and TAK-981 *in vivo*. (**F**, **G** and **H**) Melanoma tumor growth curve (**F**) and tumor mass (**G** and **H**) in nude mice at day 14 treated with DMSO and TAK-981 *in vivo*. (**I**) Western

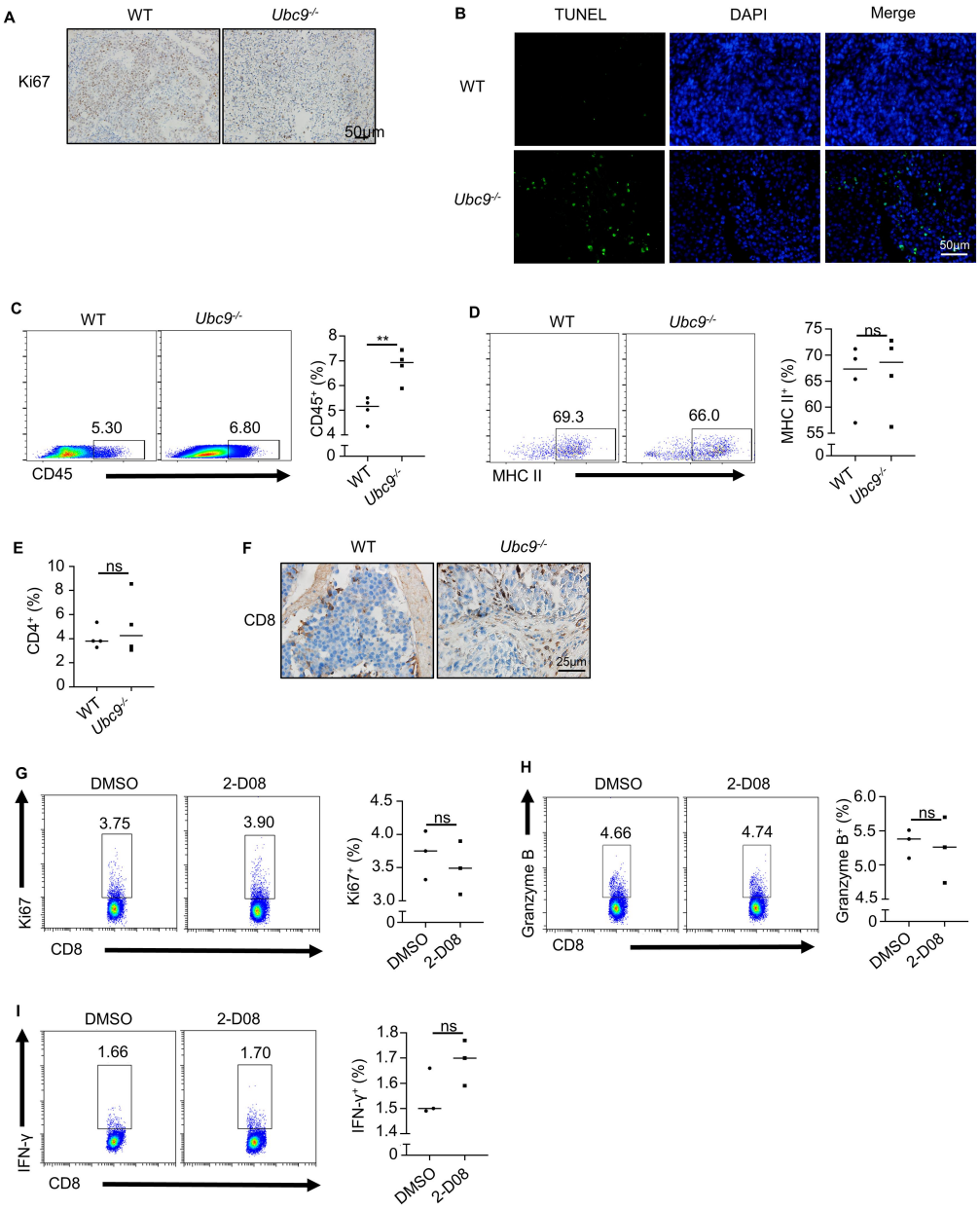
blotting assay on the RM-1 cells transduced with *Ubc9* adenovirus (*Ubc9*-OE) against vector (vector as control). (**J**, **K** and **L**) Prostate tumor growth curve (**J**) and tumor mass (**K** and **L**) of RM-1 transduced with *Ubc9*-OE or control (NC), treated with 2-D08 in nude mice at day 14 *in vivo, n* = 7 per group. Data represent mean ± SEM and were analyzed by Student's *t*-test. ***$P < 0.001$; ****$P < 0.0001$; ns, not significant.

Supplemental Figure 3.



***Ubc9* deficiency in macrophages accelerates prostate tumor apoptosis and immune cell infiltration.** (**A**) Genotyping for the *Lyzm-Cre* and *Ubc9*$^{fl/fl}$. (**B**) TUNEL staining of tumors from WT and *Ubc9*$^{-/-}$ mice. (**B, C** and **D**) CD45^{+} immune cells (**C**), MHC II^{+} macrophages (**D**), and CD4^{+} T cells (**E**) in tumors from WT and *Ubc9*$^{-/-}$ mice, respectively. (**F**) IHC staining of CD8^{+} T cells in the two groups. Representative data from three experiments are shown for (**B**) and (**F**). Data in (**C-E**) represent mean ± SEM and were analyzed by Student's *t*-test (n = 4 / group). ***P < 0.001; ns, not significant.

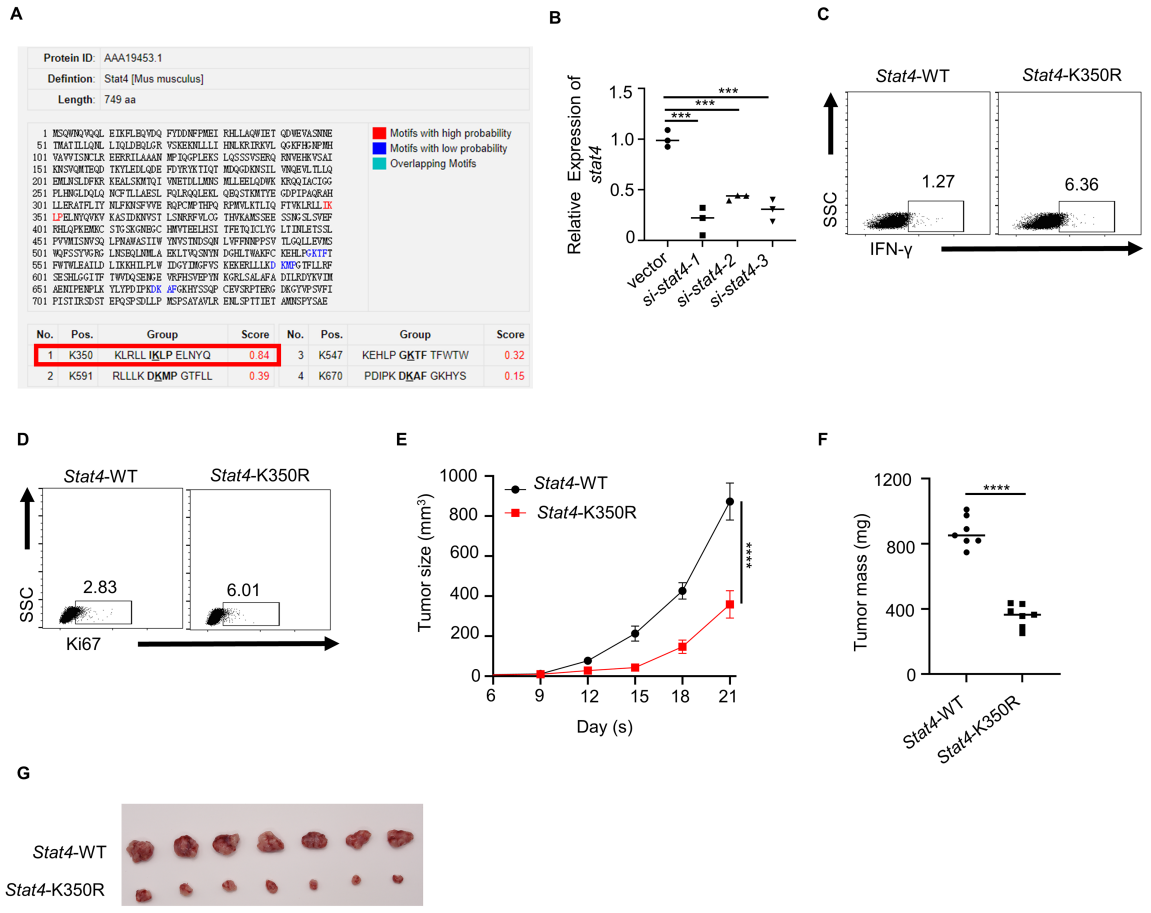
Supplemental Figure 4.



***Ubc9* deficiency in macrophages promotes CD8⁺ T cell infiltration while 2-D08 treated tumor cells fail to enhance CD8⁺ T cell activation. (A** and **B)** Ki67 staining (**A**) and TUNEL staining (**B**) of B16-OVA tumors adjacently transferred with OVA-pulsed WT or *Ubc9*$^{-/-}$ macrophages, *n* = 4 per group. (**C**, **D** and **E**) CD45⁺ immune cells (**C**), MHC II⁺ macrophages (**D**), and CD4⁺ T cells (**E**) in tumors from these two groups, respectively, *n* = 4 per group. (**F**) IHC staining of CD8⁺ T cells in the two groups. (**G**, **H** and **I**) The proportions of Ki67⁺ (**G**), Granzyme B⁺ (**H**) and IFN-γ⁺ (**I**)

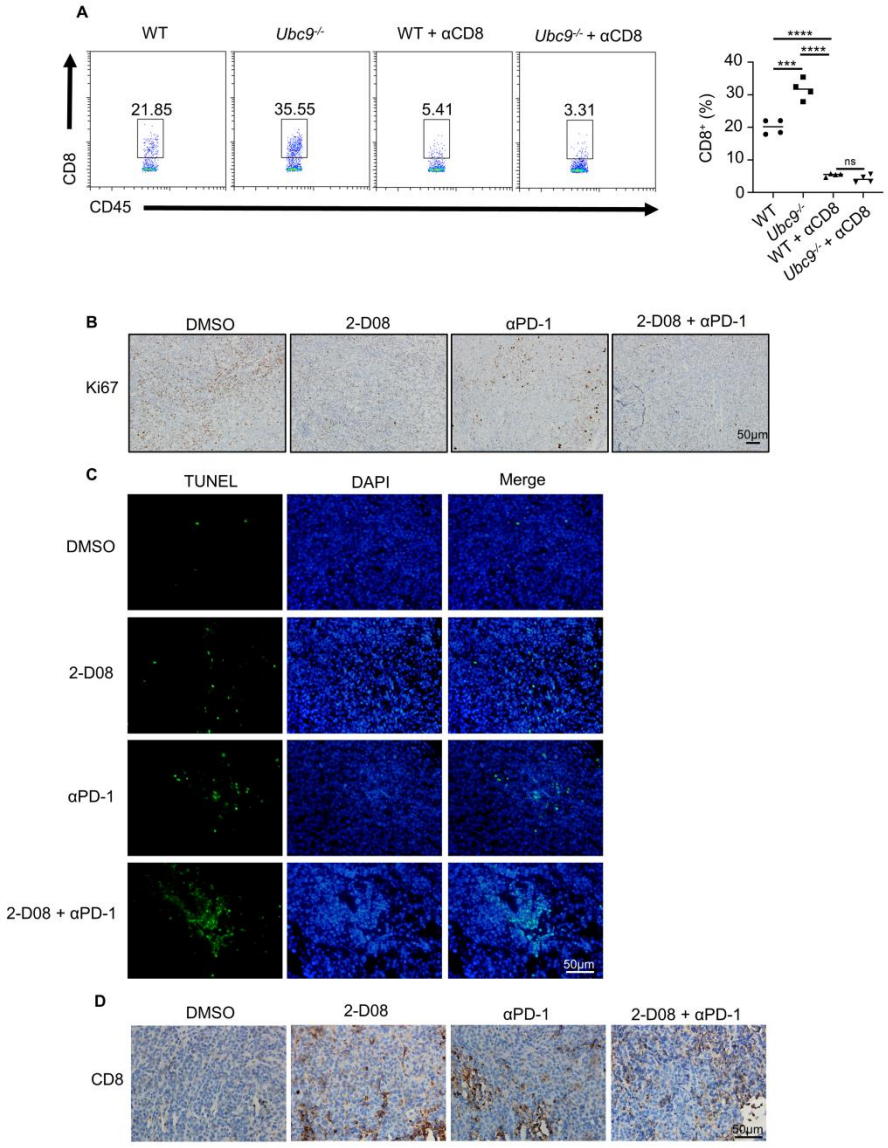
$CD8^+$ T cells in co-culture assay combining B16-OVA pre-treated with DMSO or 2-D08 with OT-I $CD8^+$ T cells, n = 3 per group. Representative data from three experiments are shown for (**A**), (**B**) and (**F**). Data in (**C-E**) and (**G-I**) represent mean ± SEM and were analyzed by Student's t-test. $^{**}P < 0.01$; ns, not significant.

Supplemental Figure 5.



SUMOylation of STAT4 at lysine residue 350 is related to the antitumor function of macrophages. (**A**) SUMOsp2.0 software predicting lysine residue 350 (K350) as a potential site of SUMOylation. (**B**) Endogenous *Stat4* in BMDMs was knocked down by siRNA. (**C** and **D**) Macrophages transduced with *Stat4*-WT or *Stat4*-K350R after endogenous *Stat4* knockdown were co-cultured with $CD8^+$ T cells in the presence of anti-CD3. The representative flow cytometry plots for IFN-γ (**C**) and Ki67 (**D**) in $CD8^+$ T cells were showed. (**E**, **F** and **G**) *Stat4*-WT or *Stat4*-K350R transduced macrophages were adoptively transferred into the adjacent sites of prostate tumors. RM-1 prostate tumor growth curve (**E**) and tumor mass (**F** and **G**) at day 14 in *Stat4*-WT and *Stat4*-K350R groups, $n = 7$ per group. (**E**) was determined by log-rank test. Data in (**B**) and (**F**) represent mean ± SEM and were analyzed by Student's *t*-test. ***$P < 0.001$; ****$P < 0.0001$; ns, not significant.

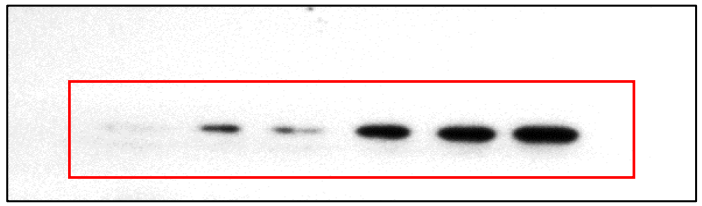
Supplemental Figure 6.



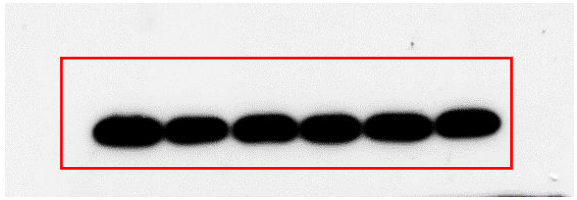
2-D08 exhibited synergistic effect with anti-PD-1 on the inhibition of prostate tumor growth. (**A**) The proportion of $CD8^+$ T cells among $CD45^+$ immune cells following $CD8^+$ T cell depletion in WT and *Ubc9*$^{-/-}$ mice, $n = 4$ per group. (**B**, **C** and **D**) Ki67 staining (**B**), TUNEL staining (**C**) and IHC staining for $CD8^+$ T cells (**D**) in the tumors from DMSO, 2-D08, anti-PD-1 antibody, or 2-D08 plus anti-PD-1 antibody groups, respectively. Data in (**A**) represent mean ± SEM and were analyzed by Student's *t*-test. ***$P < 0.001$; ****$P < 0.0001$; ns, not significant.

Full unedited gel for Figure 1G

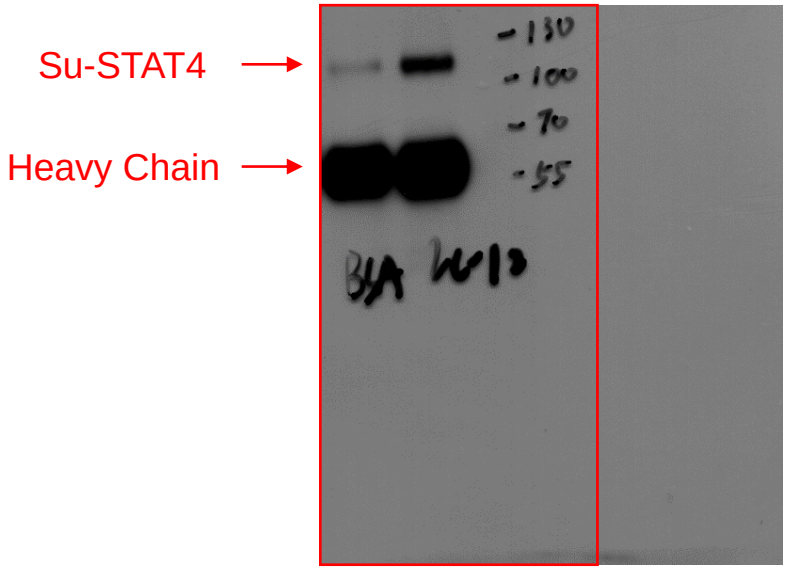
UBC9



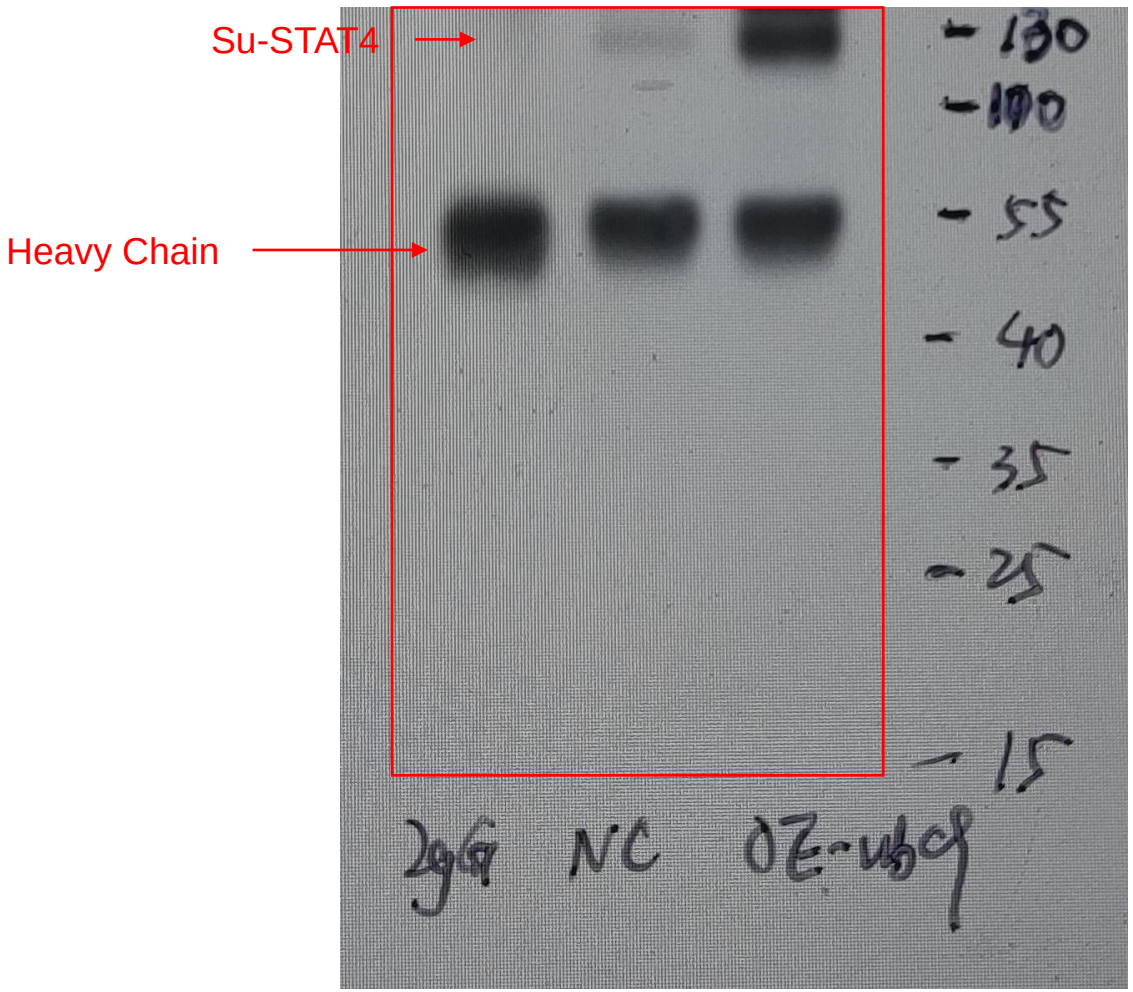
β-actin



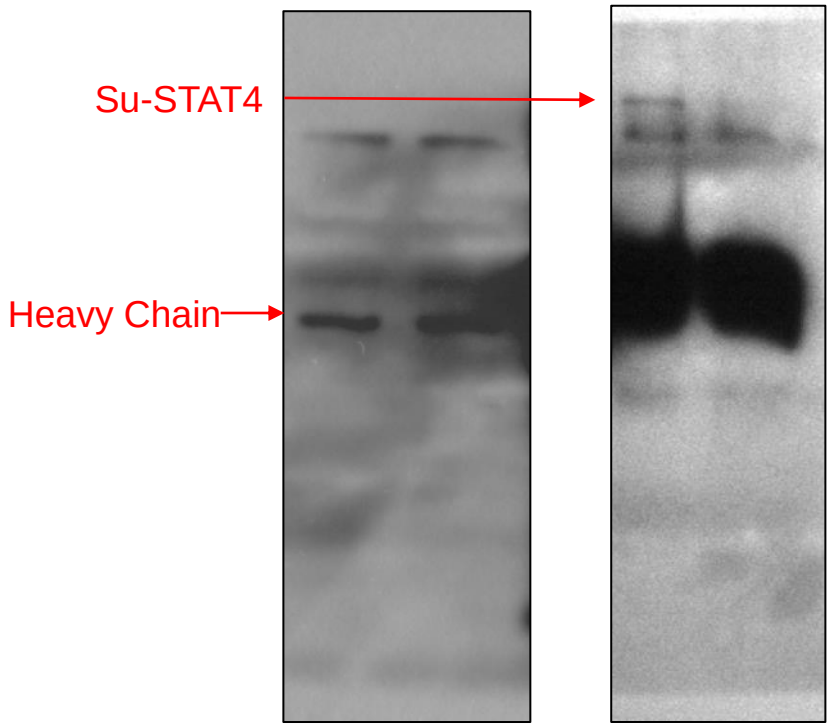
Full unedited gel for Figure 6C



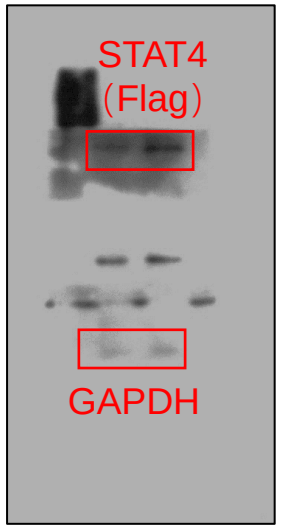
Full unedited gel for Figure 6D

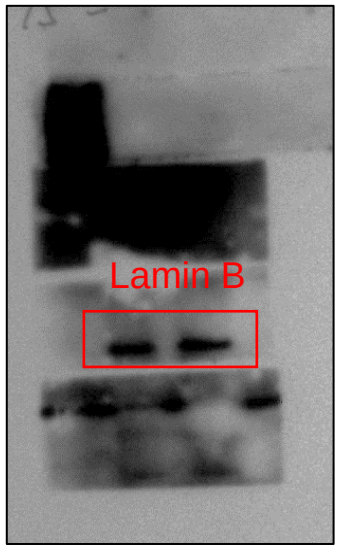


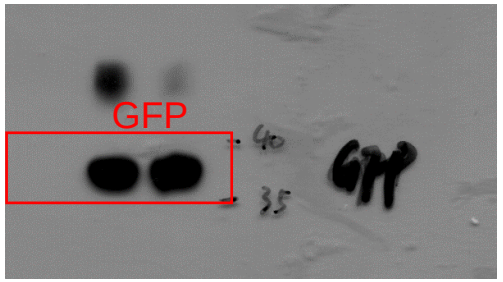
Full unedited gel for Figure 6E



Full unedited gel for Figure 6F







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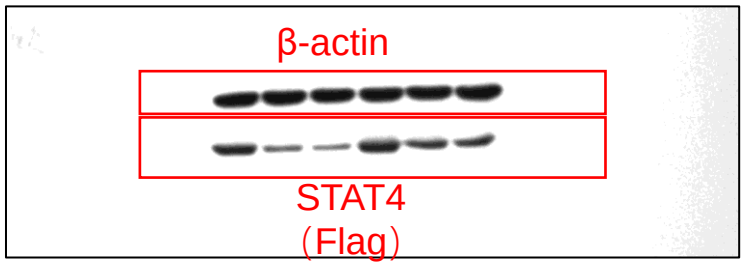
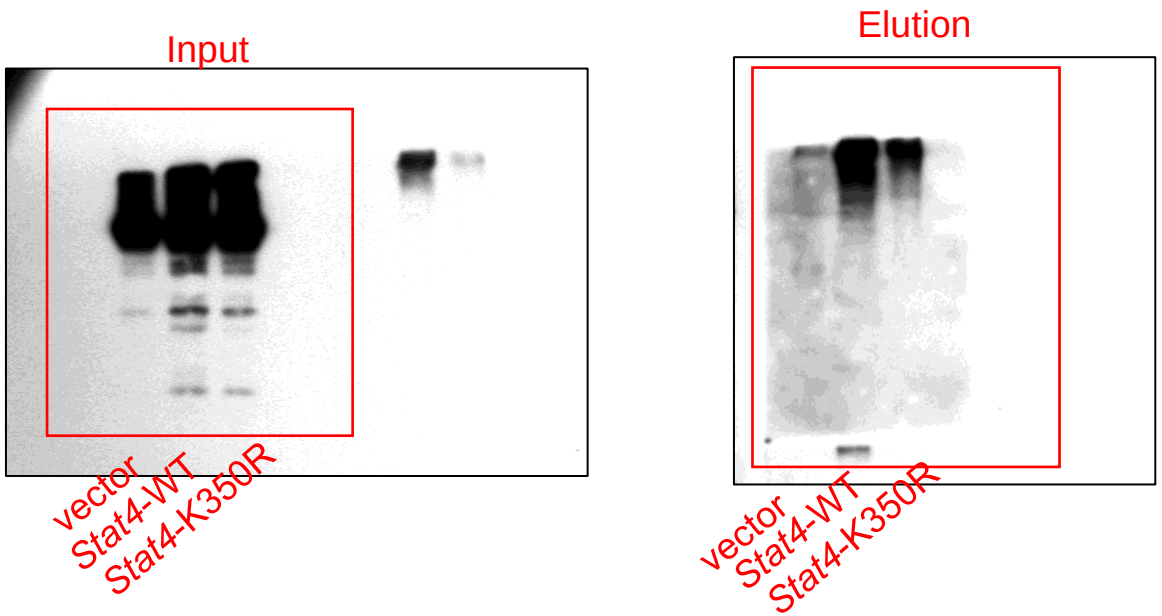
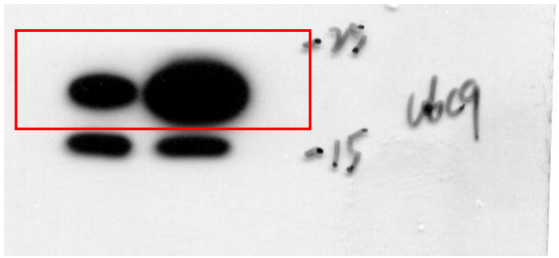


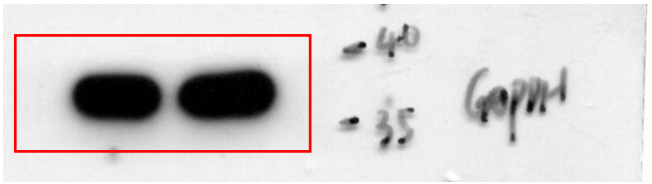
Full unedited gel for Figure 6I



Full unedited gel for Supplementary Figure 2I

UBC9





GAPDH