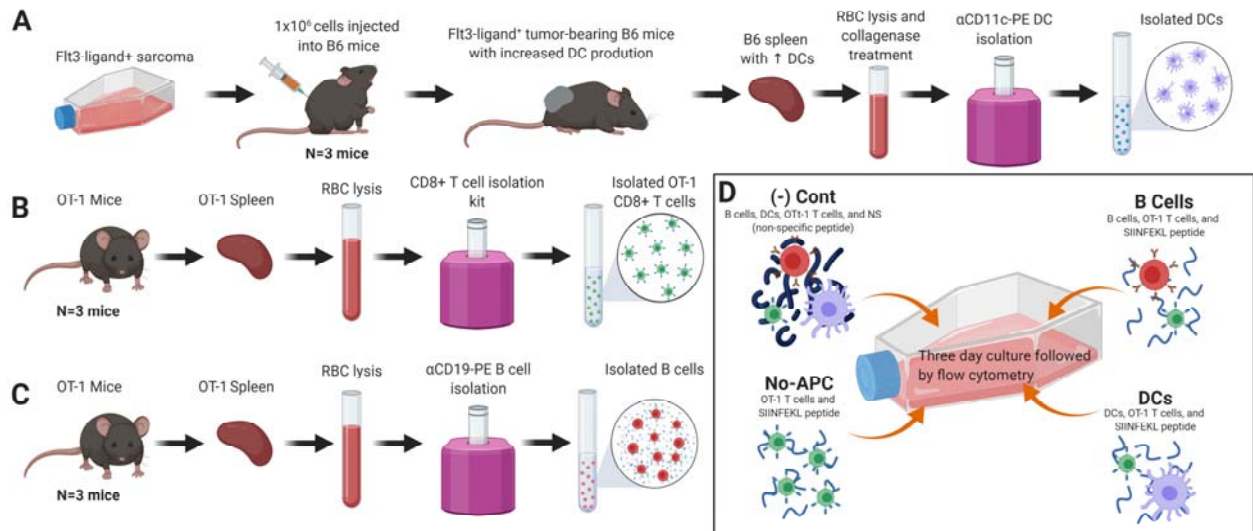
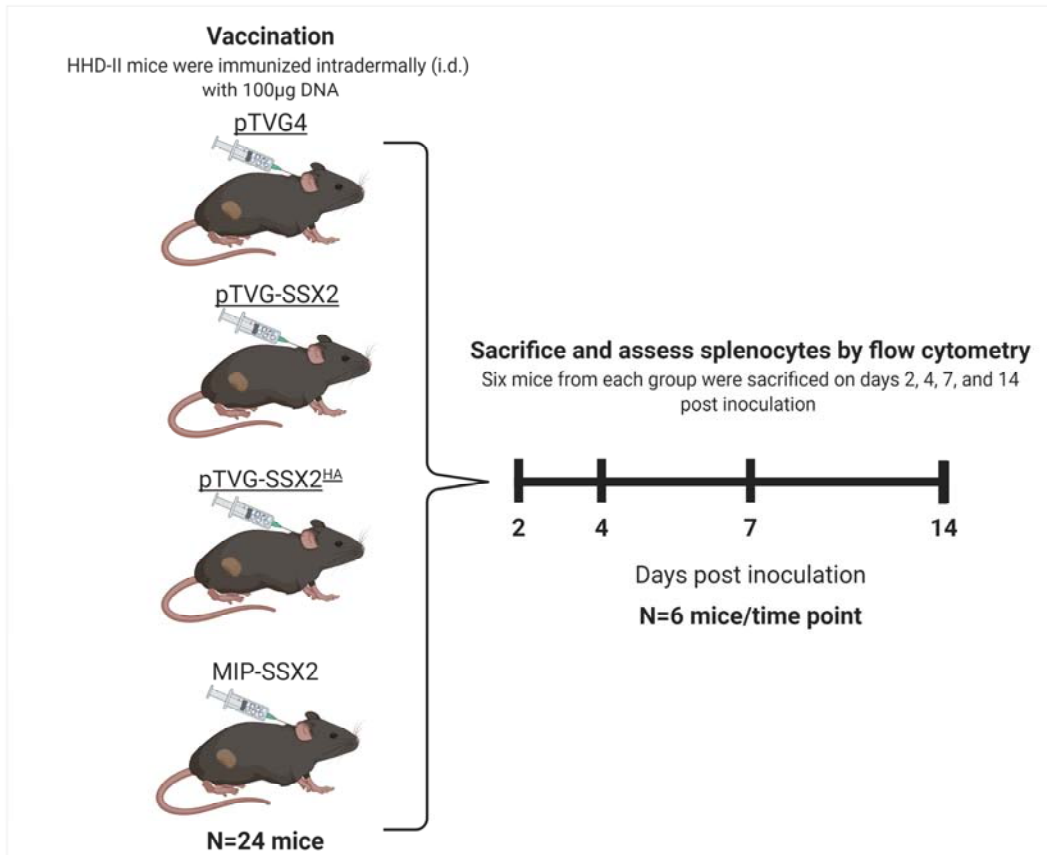


## Co-culture experiments



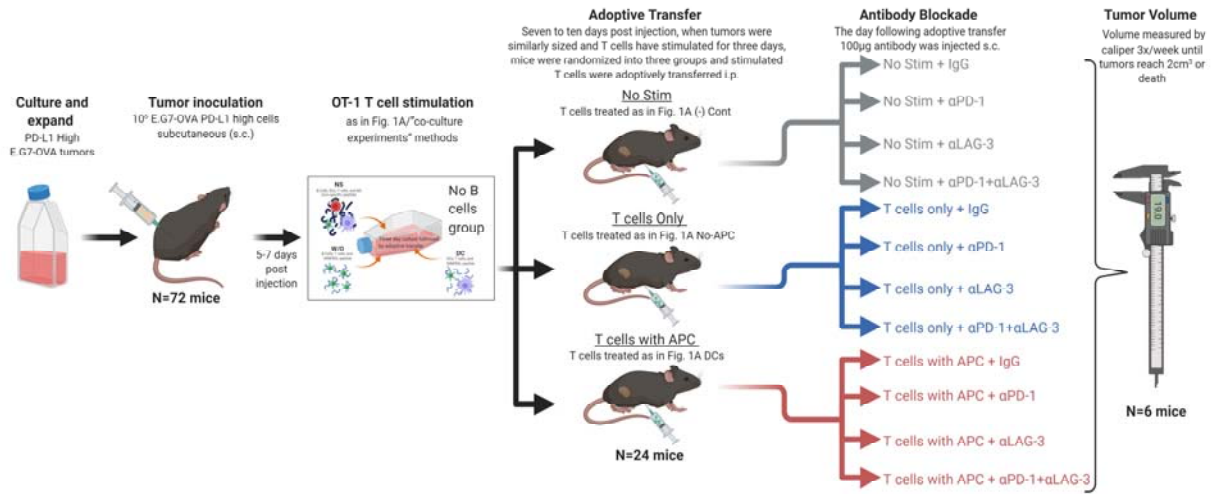
**Supplemental Figure S1 – Co-culture experimental methods schematic.** Shown in A-C are flow diagrams for the isolation/purification of DCs (A), T cells (B) and B cells (C). Panel D indicates which cells were cultured together for the studies described in relation to Figure 1.

## Vaccination studies



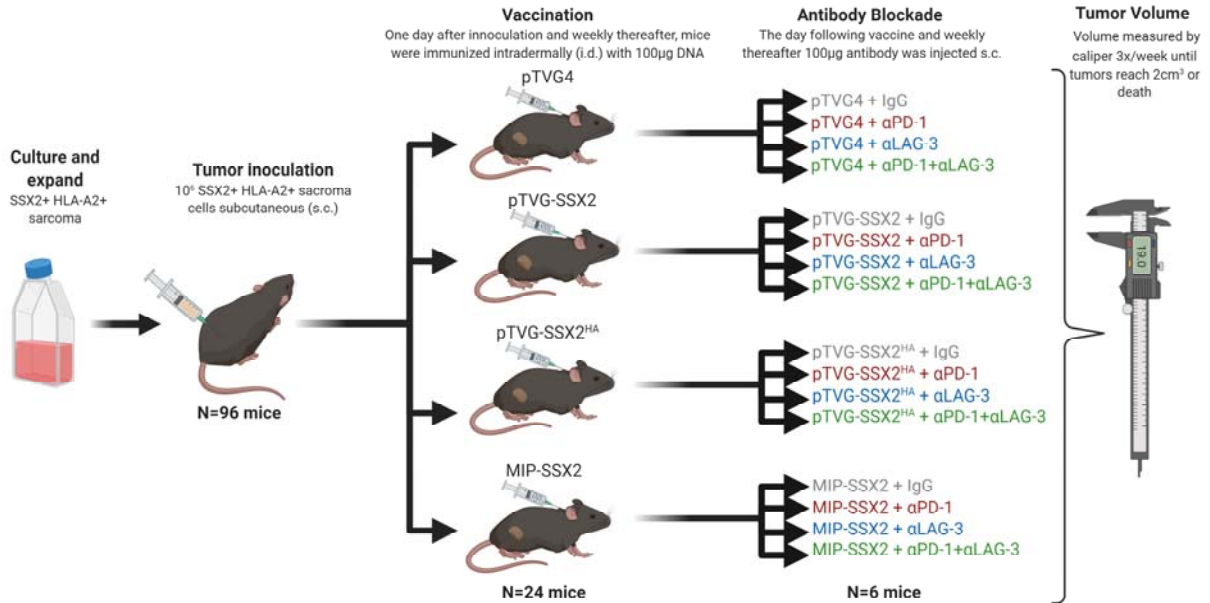
**Supplemental Figure S2 – Immunization studies in HHD-II mice.** Shown is a schematic flow diagram of the experiments conducted using SSX2-targeted DNA vaccines in HHD-II mice.

## Tumor treatment studies: *E.G7-OVA* tumors in *B6* mice



**Supplemental Figure S3 – Tumor treatment studies: *E.G7-OVA* tumors in *B6* mice.** Shown is a schematic flow diagram for the studies using adoptive transfer of T cells to *E.G7-OVA* tumor-bearing mice.

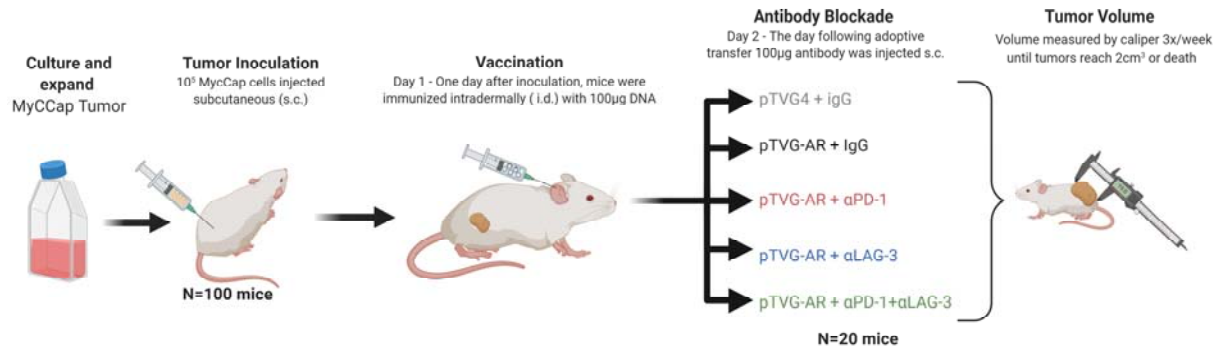
## Tumor treatment studies: *SSX2* sarcomas in HHD (*HAL-A2*) mice



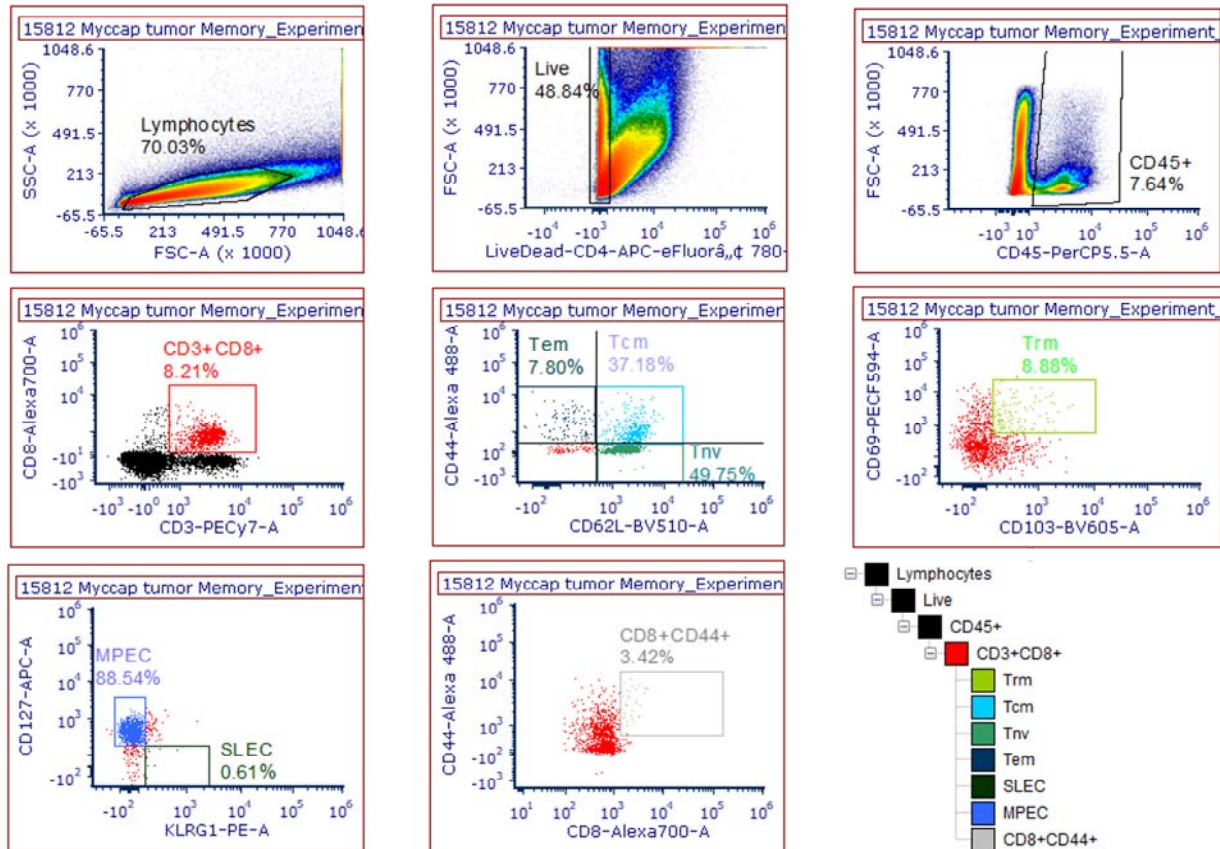
### Supplemental Figure S4 – Tumor treatment studies: SSX2+ sarcomas in HHD-II mice.

Shown is a schematic flow diagram of the experiments conducted using SSX2+ sarcomas in HHD-II mice.

## Tumor treatment studies: *Myc-CaP tumors in FVB mice*



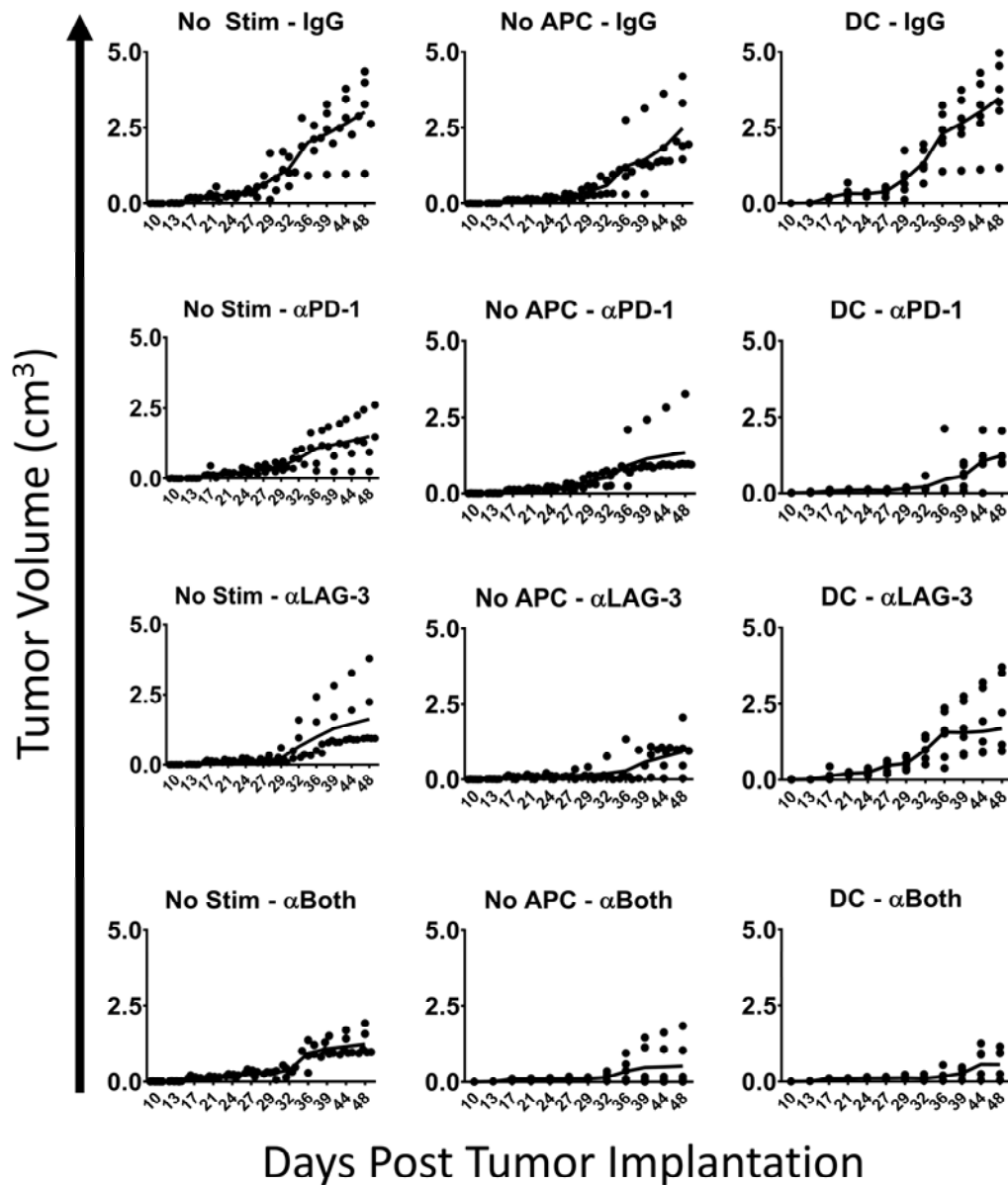
**Supplemental Figure S5 – Tumor treatment studies: MycCaP tumors in FVB mice.** Shown is a schematic flow diagram of the experiments conducted using DNA vaccines with T-cell checkpoint blockade in MycCaP tumor-bearing FVB mice.



**Supplemental Figure S6 – Gating strategy for tumor-infiltration T cells (TIL) analysis.**

Shown is the flow cytometry gating strategy employed to assess TIL and memory phenotypes.

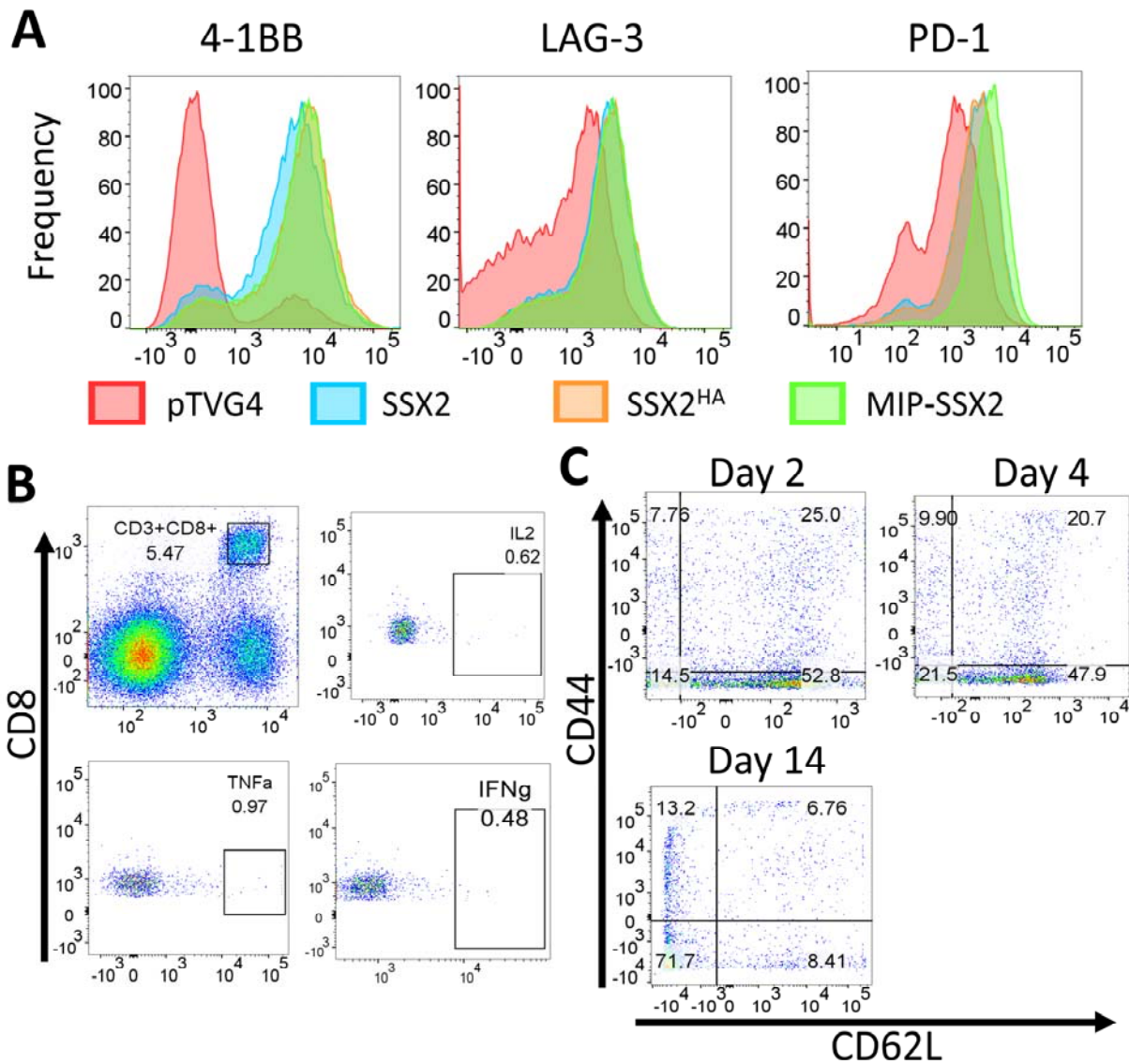
From left to right, top to bottom, all cells were evaluated by forward and side scatter to include lymphocytes, gated for live events, then gated for CD45 expression, then CD3+CD8+ cells were gated for the expression of memory markers as shown.



**Supplemental Figure S7 – Individual growth curves for Figure 2, OVA-expressing tumor study.** B6 mice were inoculated with  $1 \times 10^6$  PD-L1-expressing E.G7-OVA cells. After ten days,  $1 \times 10^6$  OT-1 T cells, stimulated with or without peptide and with or without APC as in Figure 1, were adoptively transferred into the tumor-bearing mice. The following day, mice were treated with IgG isotype control (top row), PD-1 blocking (second row), LAG-3 blocking (third row), or

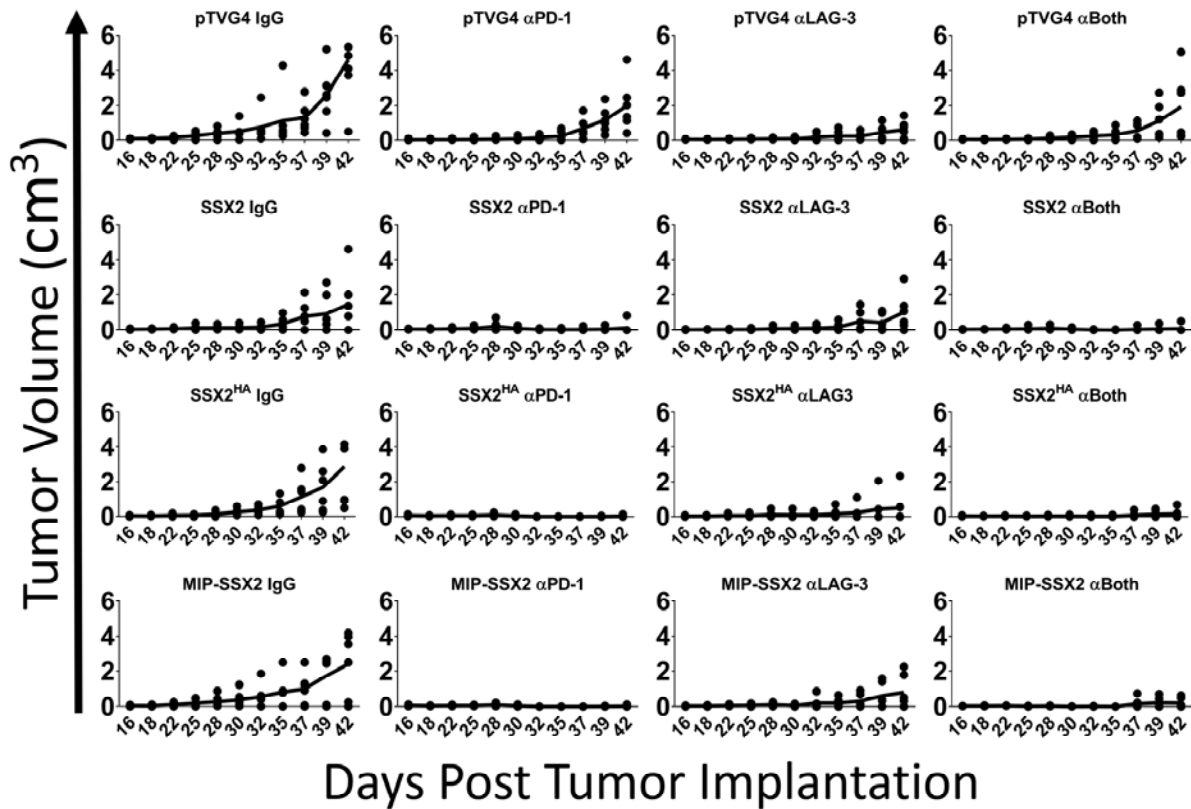
a combination of both PD-1 and LAG-3 blocking antibodies (fourth row). Tumor growth was measured as indicated on the X axes. Shown are the individual tumor measurements for each mouse per day following tumor implantation, and the median tumor size. Results are from one experiment with N=6 mice per group.



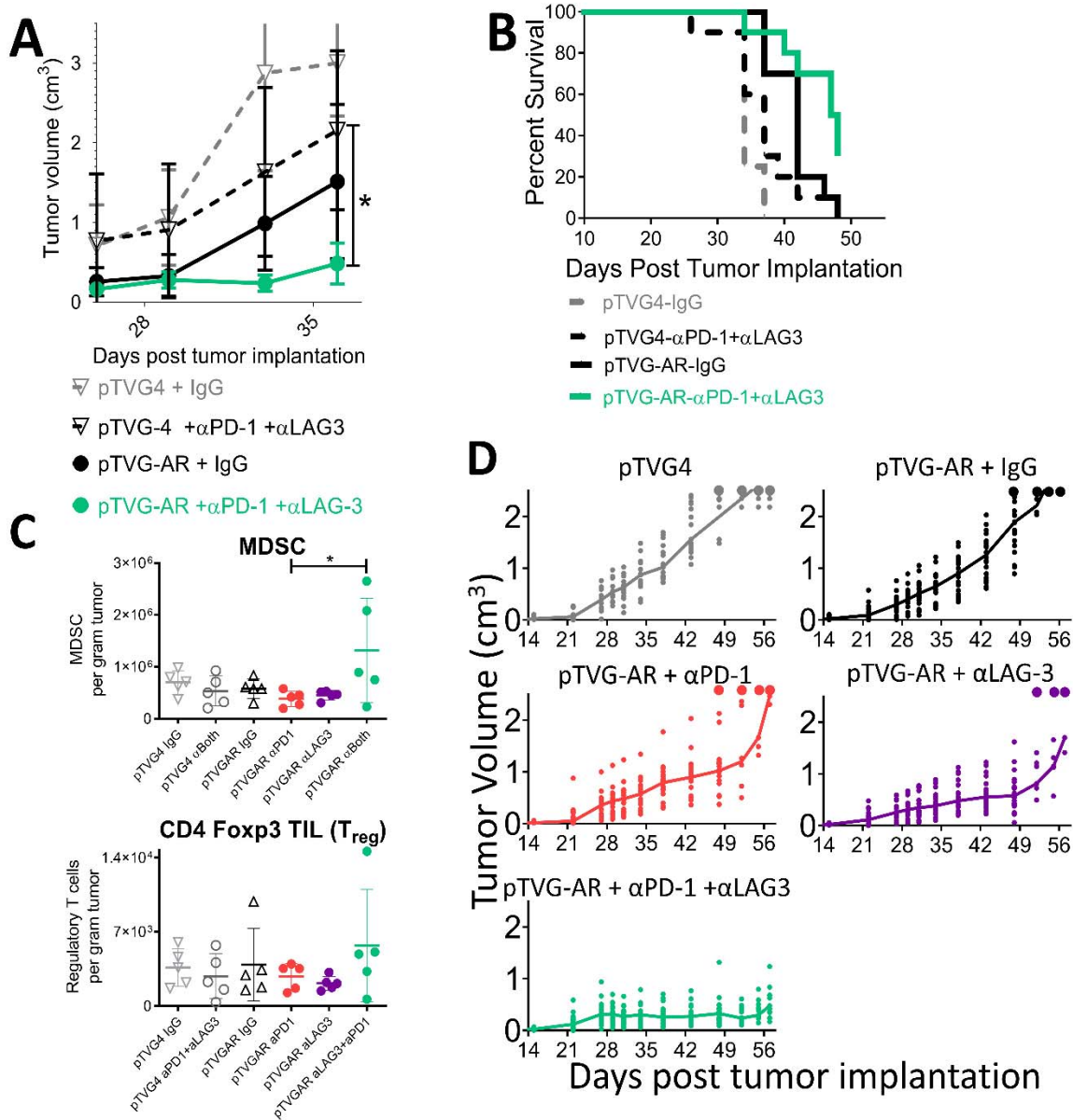


**Supplemental Figure S8 – Representative histograms and dot plots for Figure 3.** Six-week-old HHDII HLA-A2<sup>+</sup> mice were immunized with pTVG4 empty vector, the native pTVG-SSX2 DNA vaccine, pTVG-SSX2<sup>HA</sup>, or MIP-SSX2. Splenocytes obtained from mice at different time points were assessed by flow cytometry for expression of various markers, as described in Figure 3. Shown are representative data for expression of 4-1BB, LAG-3, and PD-1 expression four days

following treatment with the different vaccines (panel A). Panel B shows representative dot plots for the intracellular cytokine analysis. The upper left plot shows the gating of live, lymphocyte scatter for CD3 (X axis) by CD8 (Y axis). The indicated gate was used to evaluate expression of the individual cytokines as shown in the other plots. Panel C shows the evaluation for CD44 and CD62L expression gated on live/CD3+/CD8+/tetramer+ cells. Quadrants are based on FMO gating performed at each time point.



**Supplemental Figure S9 – Individual growth curves for Figure 4, SSX2/HLA-A2 tumor study.** Six week-old HHDII (HLA-A2<sup>+</sup>) mice were inoculated s.c. with SSX2<sup>+</sup> HLA-A2<sup>+</sup> sarcoma cells and immunized with pTVG4 empty vector (top row), pTVG-SSX2 (second row), pTVG-SSX2<sup>HA</sup> (third row), or MIP-SSX2 (fourth row) in combination with IgG control (first column), αPD-1 (second column), αLAG-3 (third column), or both αPD-1/αLAG-3 (fourth column), as described in Figure 4. Tumor growth was measured over time. Shown are the individual tumor measurements for each animal per group and the median (line) for each treatment.



**Supplemental Figure S10 – Vaccination with PD-1 and LAG-3 blockade is superior to antibody treatment alone in MycCaP prostate tumors.** Six-week-old FVB mice ( $n=10$  per group) were inoculated s.c. with  $10^6$  MyC-CaP cells and immunized with pTVG4 empty vector or pTVG-AR in combination with IgG control or  $\alpha$ PD-1 and  $\alpha$ LAG-3 antibodies. Tumor growth was measured over time (panel A). Animals with tumors greater than  $2 \text{ cm}^3$  in size were euthanized,

and data are censored at 2 cm<sup>3</sup>. Kaplan-Meier curves depicting either the time of death or when the tumor reached 2 cm<sup>3</sup> in size, whichever occurred first (panel B). Panel C: In a separate study, six-week-old FVB mice (n=5 per group) were inoculated s.c. with 10<sup>6</sup> MyC-CaP cells and immunized the following day and weekly with pTVG4 empty vector or pTVG-AR. Groups received IgG control,  $\alpha$ PD-1,  $\alpha$ LAG-3, or both  $\alpha$ PD-1 and  $\alpha$ LAG-3 antibodies the day after each immunization. On day 29, tumors were collected, digested with collagenase, and evaluated for the presence of CD11b+Gr-1+ (MDSC) cells or CD4+FoxP3+ (Treg) among live cells. These are expressed as an absolute number per gram of tumor. Panel D: Individual growth curves for mice from Figure 5A. Asterisks in panels A and C indicate p<0.05 assessed by the mixed-effects model with Geisser-Greenhouse correction or two-way ANOVA, both with Tukey's multiple comparisons test with individual variances.