Supplementary Materials for

PD-1 blockade unleashes effector potential of both high and low affinity T-cells infiltrating tumors

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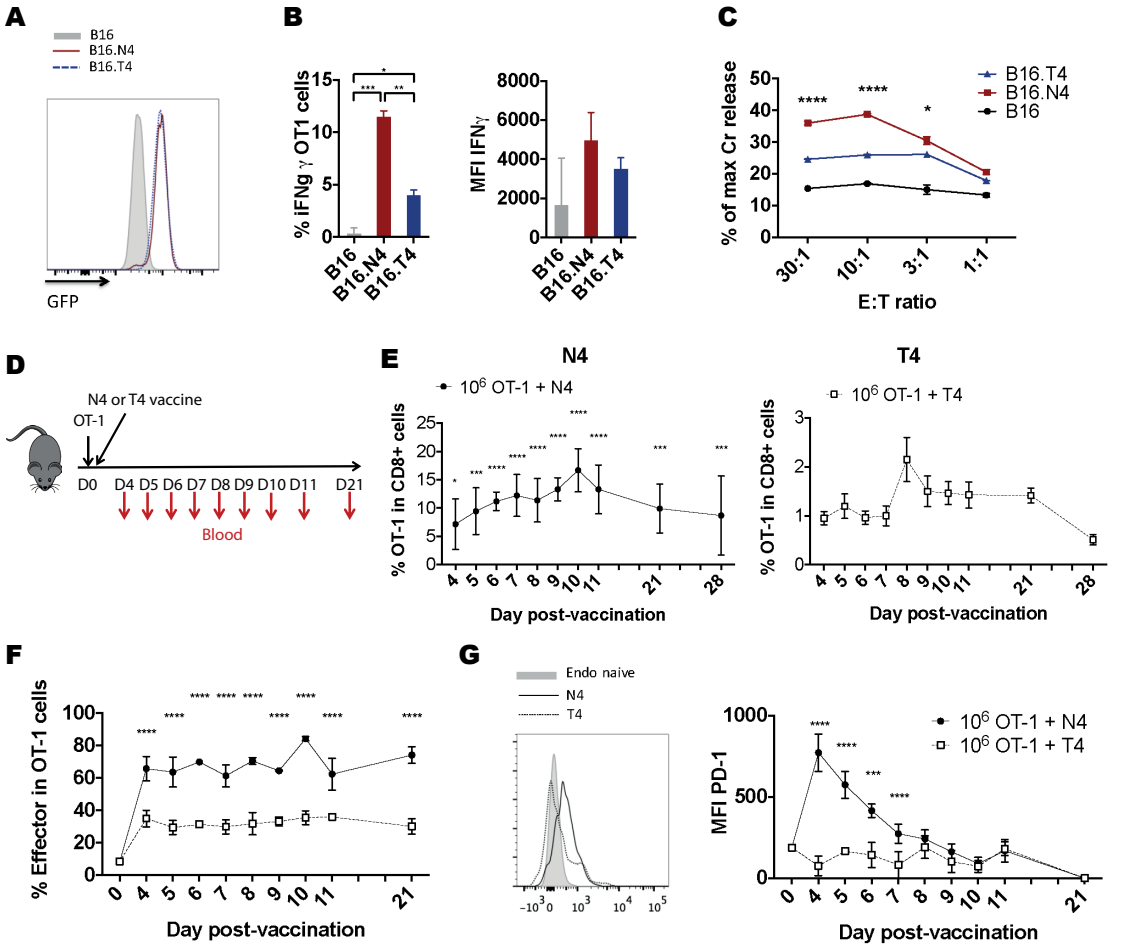

Figure S1. OT-1 cells respond to B16.T4 with lower avidity than to B16.N4 cells. A. B16.N4 and B16.T4 cell lines express similar antigen levels. Analysis of GFP expression by flow cytometry of B16.N4 and B16.T4 cell lines. **B.** Percentage of IFNγ^{+} OT-1 cells and IFNγ MFI of IFNγ^{+} OT-1 cells after *in vitro* stimulation of OT-1 cells with B16.N4 or B16.T4 cell lines. Bars represent the mean + SD (n=2). **C.** Analysis of OT-1-mediated B16.N4 and B16.T4 target cell lysis by chromium release assay. Dots represent the mean ± SD of the percentage of maximum Cr^{51} release (n=2). **D. Low compared to high affinity vaccination leads to earlier but decreased expansion and CD8^{+} T-cell effector differentiation.** Experimental design. 10^6 OT-1 cells were i.v. transferred to C57BL/6 mice. The same day mice were s.c. vaccinated with N4 or T4 peptide in combination with CpG. Blood was harvested every day during 11 days and on day 21 (n=5/group per timepoint). **E.** Percentage of OT-1 cells in total CD8^{+} T-cells. **F.** Percentage of effector (CD44^{high} CD62L^{-}) cells in total OT-1 cells. **G.** Representative histogram and quantification of PD-1 MFI of OT-1 cells. A 1-way ANOVA followed by Tukey's multiple comparison test was performed in panel B, a 2-way ANOVA followed by Tukey's multiple comparison test in panel C and a 2-way ANOVA followed by Sidak's multiple comparison test in panels E-G. Representative of two independent experiments.

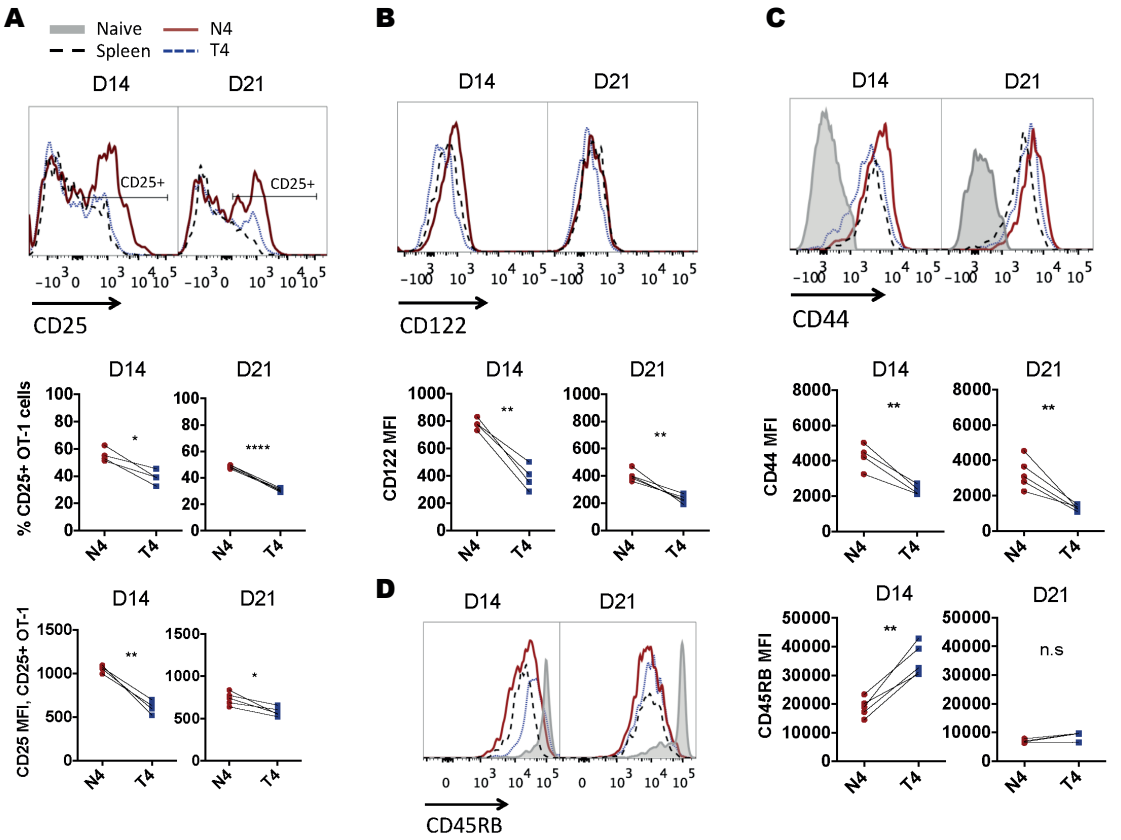

Figure S2. High affinity tumor recognition leads to increased differentiation and responsiveness to homeostatic cytokines. A, B, C and D. Representative histograms of CD25, CD122, CD44 and CD45RB in OT-1 cells from spleen (black, dashed), B16.N4 (red, continuous) and B16.T4 (blue, dotted) tumors and endogenous naïve $CD8^+$ T-cells (filled grey). Bellow quantification of each marker's MFI can be found except in figure A that percentage of $CD25^+$ cells in OT-1 cells and CD25 MFI of $CD25^+$ OT-1 cells is represented. A paired t-test or Wilcoxon test was performed after Shapiro-Wilk normality test. Representative of two independent experiments.

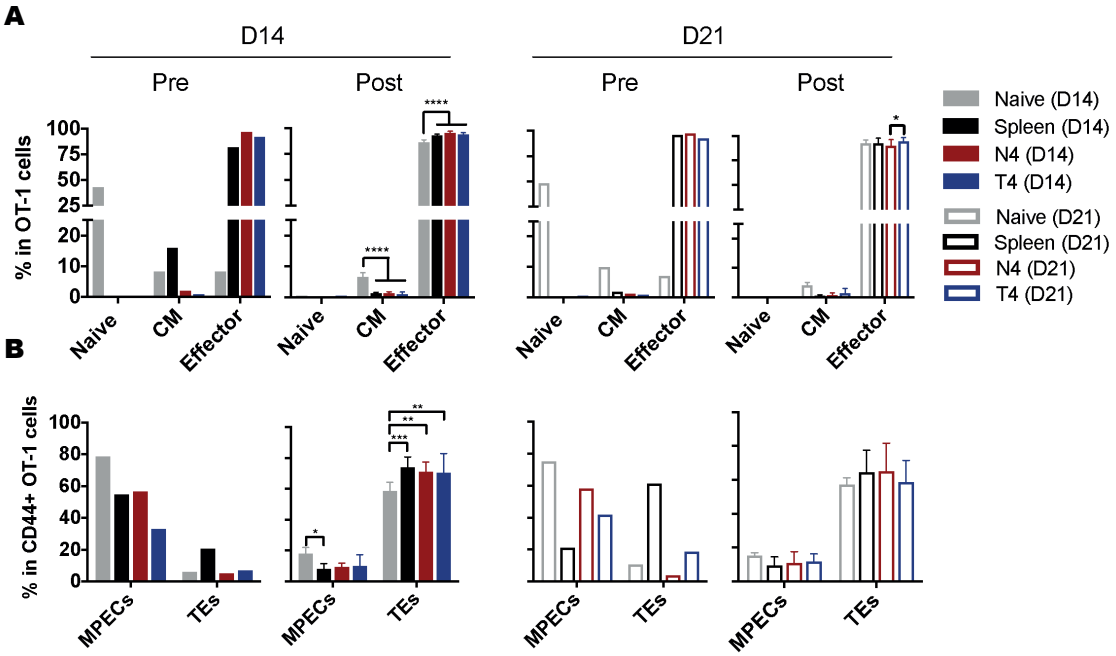

Figure S3. Loss of phenotypic differences between OT-1 cells from spleen and tumors in secondary tumor-free responses. **A.** Percentage of Naïve, CM and Effector cells in OT-1 cells 14 and 21 days post-tumor engraftment pre-transfer (Pre) and 8 days post-infection in secondary hosts (Post). **B.** Percentage of MPECs and TEs in CD44^{high} OT-1 cells 14 and 21 days post-tumor engraftment pre-transfer and 8 days post-infection in secondary hosts. A 2-way RM ANOVA followed by Tukey's multiple comparison test was performed. Bars represent the mean + SD. n=1 pre-transfer and n=8 post-infection. Representative of 2 independent experiments.

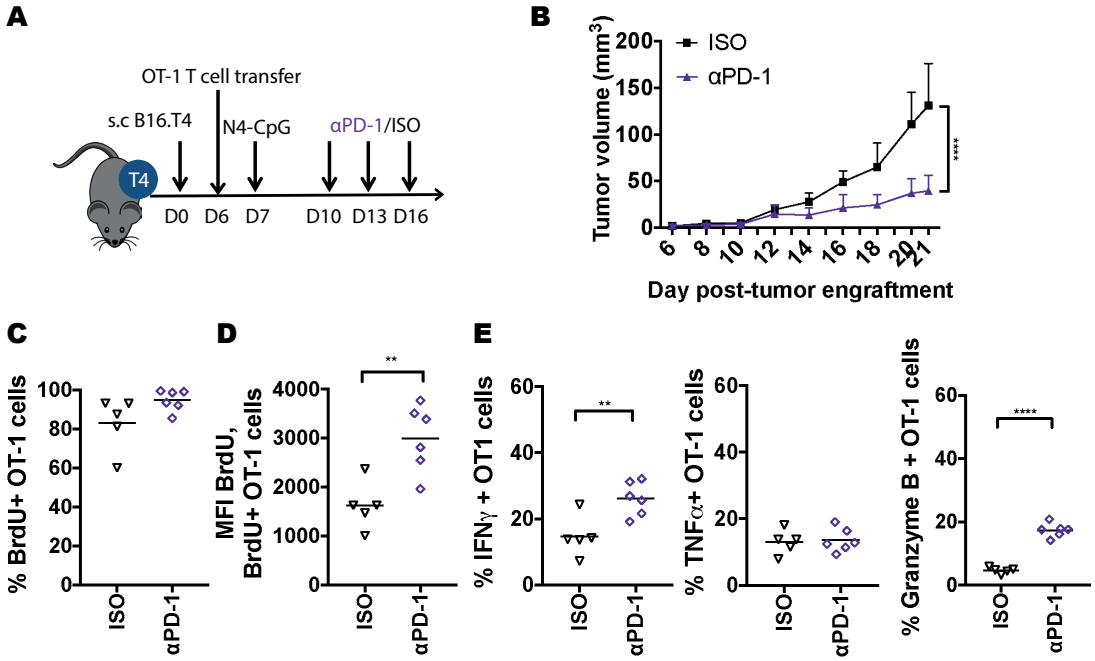

Figure S4. αPD-1 treatment enhances proliferation and cytokine production of OT-1 cells in tumors of B16.T4 tumor bearing mice. **A.** Experimental design. B16.T4 single tumor bearing mice received 10^5 OT-1 cells on day 6 and N4-CpG s.c vaccination the day after. αPD-1 or ISO control antibodies were given on day 10, 13 and 16 i.p. (n=5 in ISO and n=6 in αPD-1 group) **B.** Tumor growth curves expressed as mean tumor volume in mm^3 + SD. **C.** Percentage of $BrdU^+$ cells in OT-1 cells from B16.T4 tumors. **D.** BrdU MFI of $BrdU^+$ OT-1 cells. **E.** Percentage of $IFN\gamma^+$, $TNF\alpha^+$ and Granzyme B^+ cells in OT-1 cells from B16.T4 tumors *in vitro* restimulated with N4 peptide. Dots represent individual mice and the bar the mean. A two way-ANOVA followed by Sidak's multiple comparison test was performed in panel B and an unpaired t-test or Mann-Whitney test after Shapiro-Wilk normality test in panels C-E. In black ISO and in purple αPD-1 treated mice.