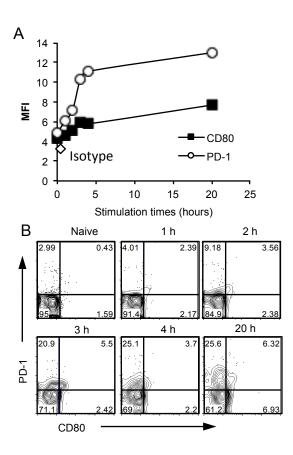
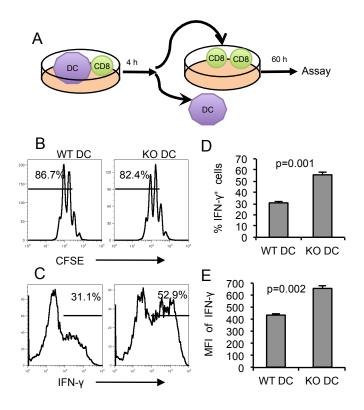
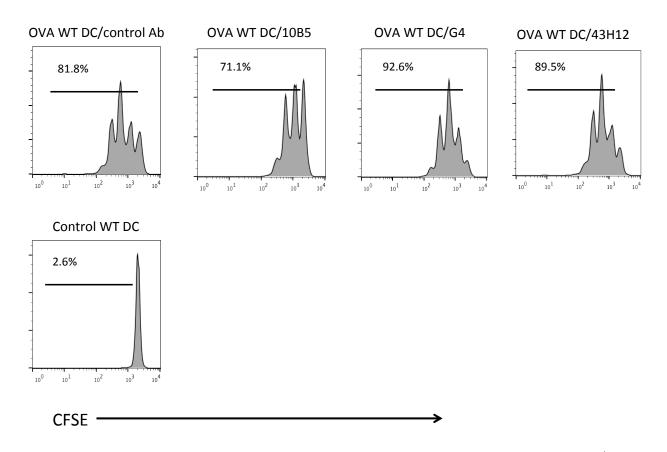
Cancer Immunology, Immunotherapy (submitted in 2014) - Rachel Gibbons et al.



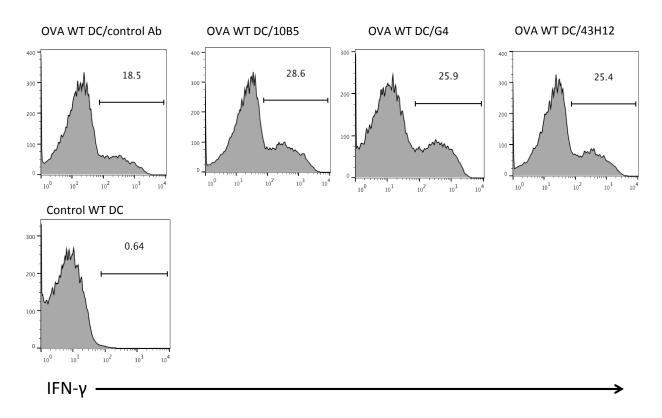
Supplemental Figure 1: Kinetics of PD-1 and CD80 expression on activated CD8⁺ T cells. Naïve OT-1 CD8⁺ T cells were primed in vitro as described in the main text with activated WT Act-mOVA dendritic cells. At the indicated time points, CD8⁺ T cells were analyzed by flow cytometry for surface expression of CD80 and PD-1. (A) Graph shows the change in mean fluorescent intensity over time after stimulation for CD80 and PD-1. (B) Dot plots show representative data obtained by flow cytometry at the indicated times after stimulation.



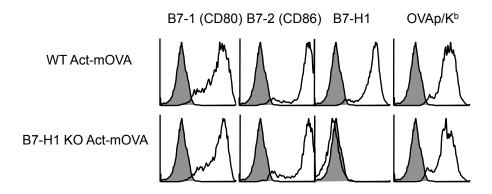
Supplemental Figure 2: CD8⁺ T cells programmed in vitro for 4 hours with B7-H1 KO dendritic cells produce more IFN- γ . CD8⁺ T cells were purified from the spleens of naïve OT-1 mice and co-cultured for 4 h with activated dendritic cells derived from the bone marrow or WT or B7-H1 KO Act-mOVA mice. CD8⁺ T cells were then reisolated from the dendritic cells and maintained in culture for 40 h. (A) Experimental design. (B) Proliferation (CFSE dilution) and (C) IFN- γ production by primed CD8 T cells was assayed by flow cytometry after a 4 h restimulation with OVA peptide. Numbers are percentages. (D) Bar graphs show the average percent of IFN- γ^+ CD8⁺ T cells and (E) levels (MFI) of IFN- γ production by CD8⁺ T cells (mean ± SD, n=3). One of three independent experiments is shown.



Supplementary Figure 3: Antibody blockade of B7-H1 during in vitro priming does not effect CD8⁺ T cell proliferation. CD8⁺ T cells were purified from the spleens of naïve OT-1 mice and co-cultured for 20 h with activated dendritic cells derived from the bone marrow WT Act-mOVA mice. The co-culture was performed in the presence of antibodies as indicated at 20 µg/ml. For a control, naïve OT-1 CD8⁺ T cells were co-cultured with activated bone marrow derived DC from a WT mouse (not Act-mOVA transgenic). CD8⁺ T cells were then re-isolated from the dendritic cells and maintained in culture for 48 h. Proliferation (CFSE dilution) production by primed CD8⁺ T cells was assayed by flow cytometry after a 4 h re-stimulation with OVA peptide. Numbers are percentages. One of three independent experiments is shown.



Supplementary Figure 4: CD8⁺ T cells programmed in vitro for 20 hours with in the presence of B7-H1 blocking antibodies produce more IFN-γ. CD8⁺ T cells were purified from the spleens of naïve OT-1 mice and cocultured for 20 h with activated dendritic cells derived from the bone marrow WT Act-mOVA mice. The co-culture was performed in the presence of antibodies as indicated at 20 µg/ml. For a control, naïve OT-1 CD8⁺ T cells were co-cultured with activated bone marrow derived DC from a WT mouse (not Act-mOVA transgenic). CD8⁺ T cells were then re-isolated from the dendritic cells and maintained in culture for 48 h. IFN-γ production by primed CD8⁺ T cells was assayed by flow cytometry after a 4 h re-stimulation with OVA peptide. Numbers are percentages. One of three independent experiments is shown.



Supplementary Figure 5: Cell surface phenotype of WT Act-mOVA and B7-H1 KO Act-mOVA activated DC. Dendritic cells were cultured from the bone marrow of WT Act-mOVA and B7-H1 KO Act-mOVA mice and activated with poly I:C. CD80, CD86, B7-H1, and OVAp/K^b expression levels were obtained by flow cytometry analysis (open lines). Filled lines are isotype controls. One of three independent experiments is shown.