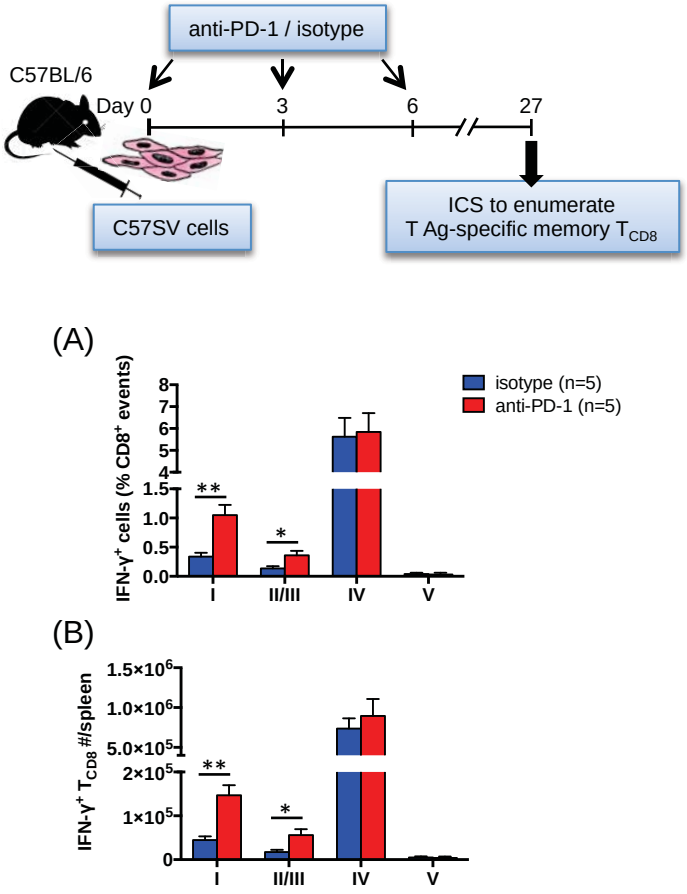
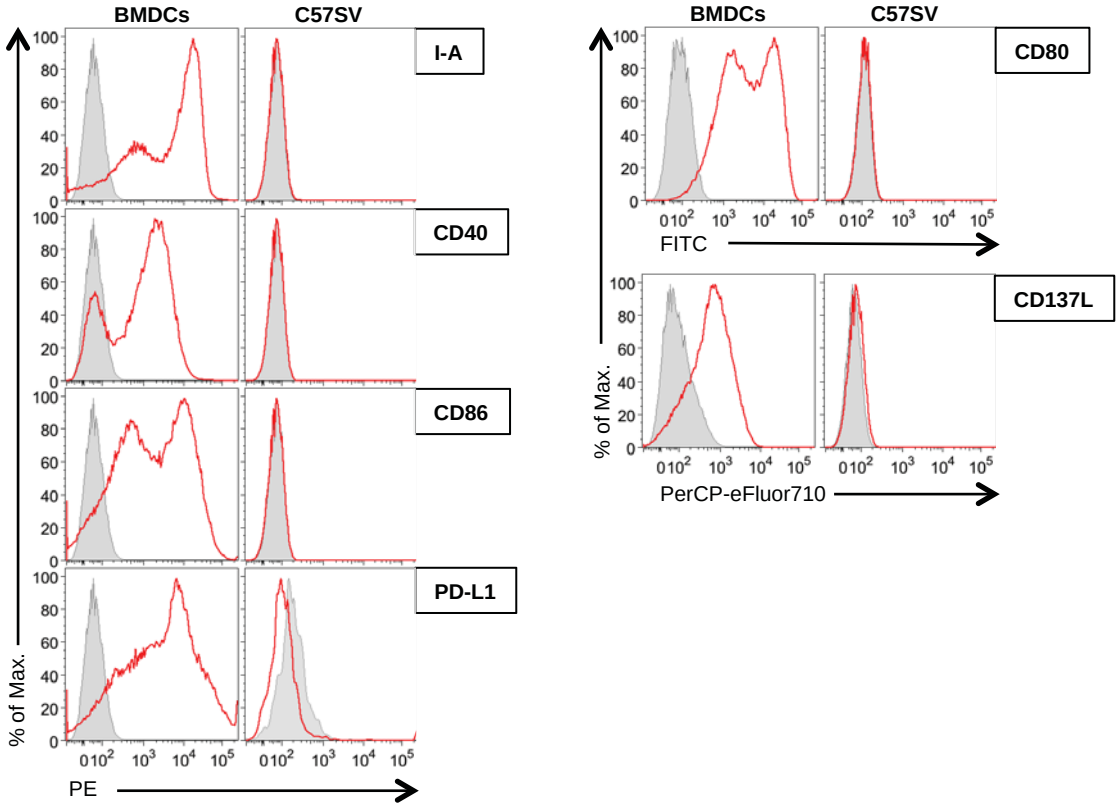
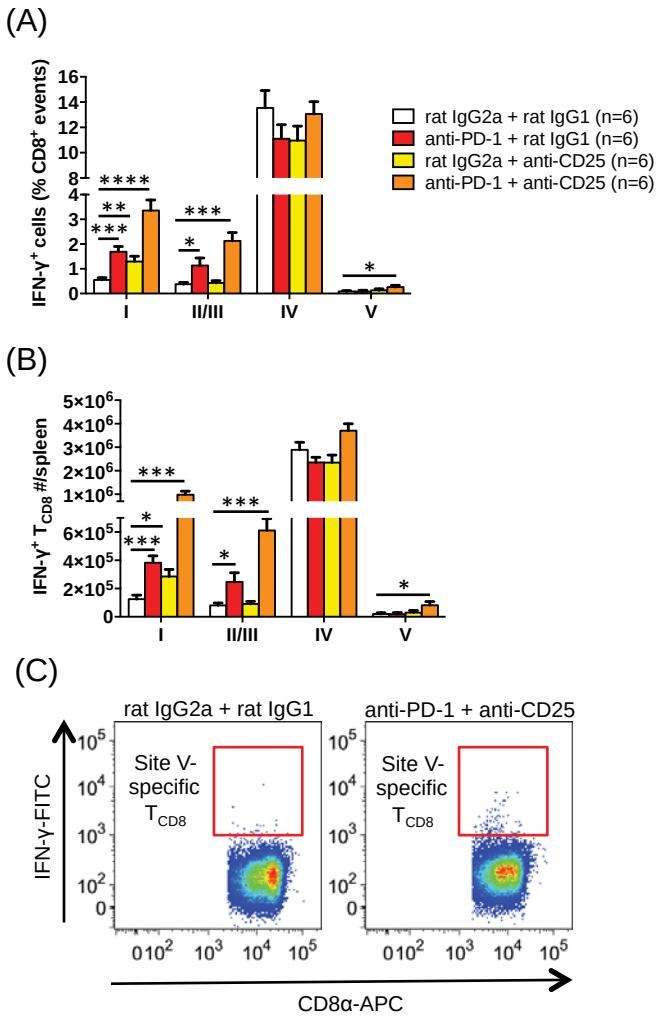


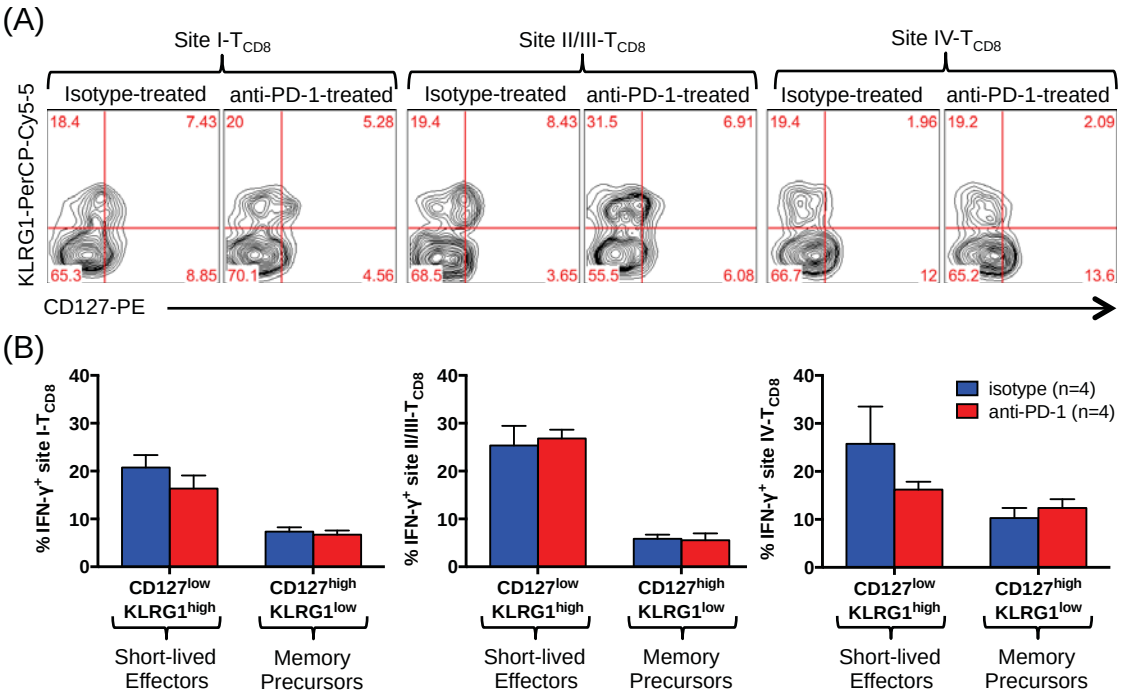
Supplemental Figure 1. PD-1 blockade increases both the frequencies and the absolute numbers of subdominant memory T_{CD8}. B6 mice were inoculated with C57SV cells and treated with 3 separate doses of an anti-PD-1 mAb or isotype control (n=5 per group). On day 27 post-priming with tumor cells, the percentages (*A*) and absolute numbers (*B*) of splenic sites I- , II/III, IV- and V-specific T_{CD8} in each spleen were determined by ICS for IFN-γ. * and ** denote statistical differences with $p<0.05$ and $p<0.01$, respectively.



Supplemental Figure 2. C57SV fibrosarcoma cells do not express MHC class II and classic costimulatory molecules or PD-L1. C57SV cells were tested by flow cytometry for surface expression of I-A, CD40, CD80, CD86, CD137L and PD-L1. Bone marrow-derived dendritic cells (BMDCs) were also stained in parallel as a positive control. Filled grey and open histograms represent staining with isotype controls and mAbs specific for indicated molecules, respectively.



Supplemental Figure 3. PD-1 blockade and nTreg cell inactivation strategies can be combined to additively enhance subdominant T Ag-specific T_{CD8} responses. B6 mice (n=6 per cohort) were injected with an anti-CD25 mAb (or a rat IgG1 isotype control) 3 days before they were inoculated with C57SV tumor cells. Mice then received separate doses of anti-PD-1 (or a rat IgG2a isotype control). Nine days later, the percentages (*A*) and absolute numbers (*B*) of splenic T Ag-specific T_{CD8} were determined by ICS for IFN-γ. *, **, *** and *** denote statistical differences with $p<0.05$ and $p<0.01$, $p<0.001$ and $p<0.0001$, respectively, and error bars represent SEM (*A*-*B*). Representative dot plots for site V-specific T_{CD8} are also presented (*C*).



Supplemental Figure 4. Short-lived effector and memory precursor frequencies of T Ag-specific T_{CD8} are comparable in anti-PD-1- and isotype-treated mice. B6 mice (n=4 per group) were inoculated with C57SV tumor cells and treated with anti-PD-1 or isotype control. Nine days later, sites I-. II/III- and IV-specific T_{CD8} were identified by ICS for IFN-γ and further evaluated for their surface expression levels of KLRG1 and CD127 (IL-7 receptor α chain). Representative dot plots are illustrated (*A*), and the percentages of CD127^{low}KLRG1^{high} short-lived effectors and CD127^{high}KLRG1^{low} memory precursors were determined within each T_{CD8} population (*B*). Error bars represent SEM.