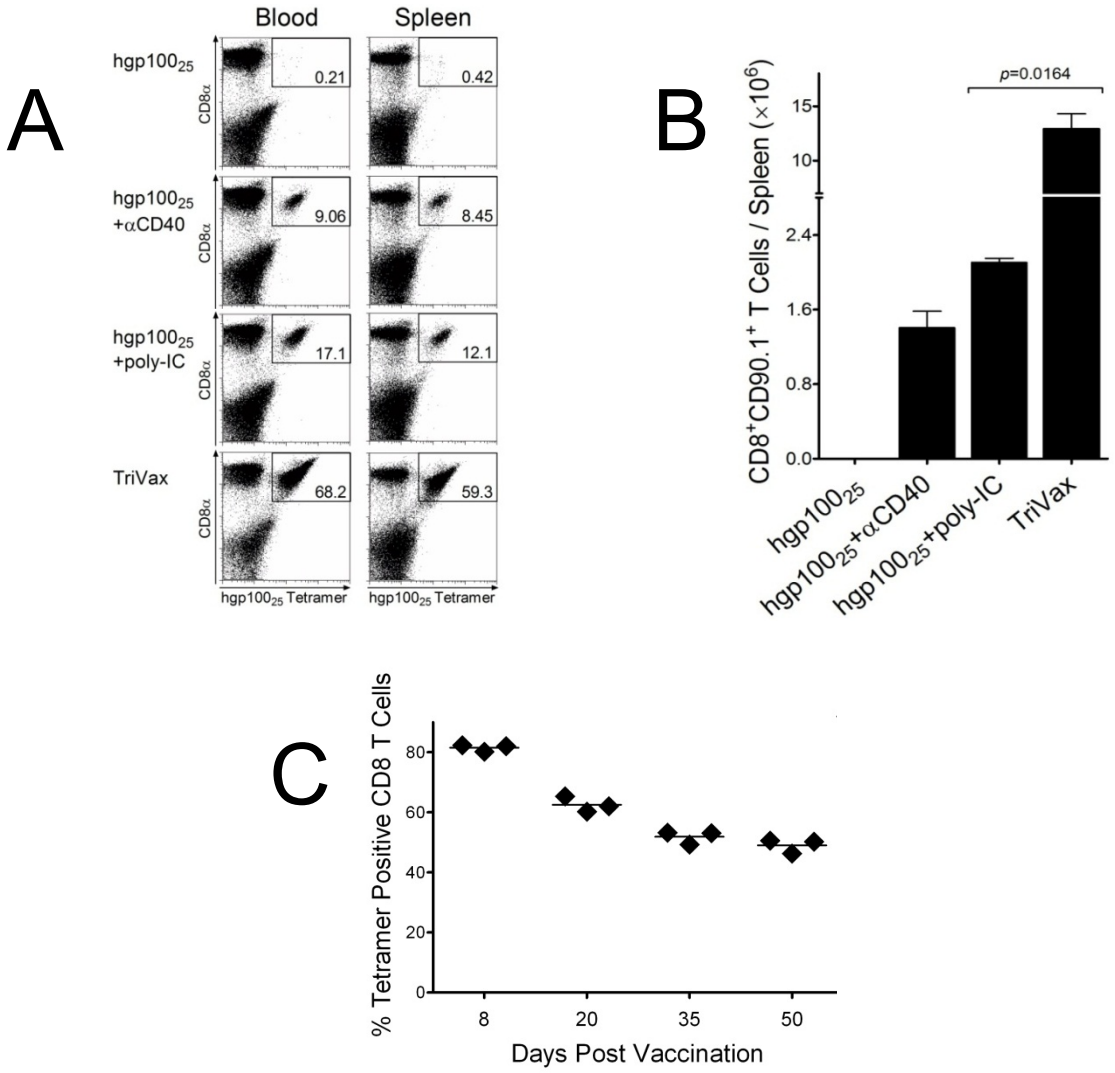
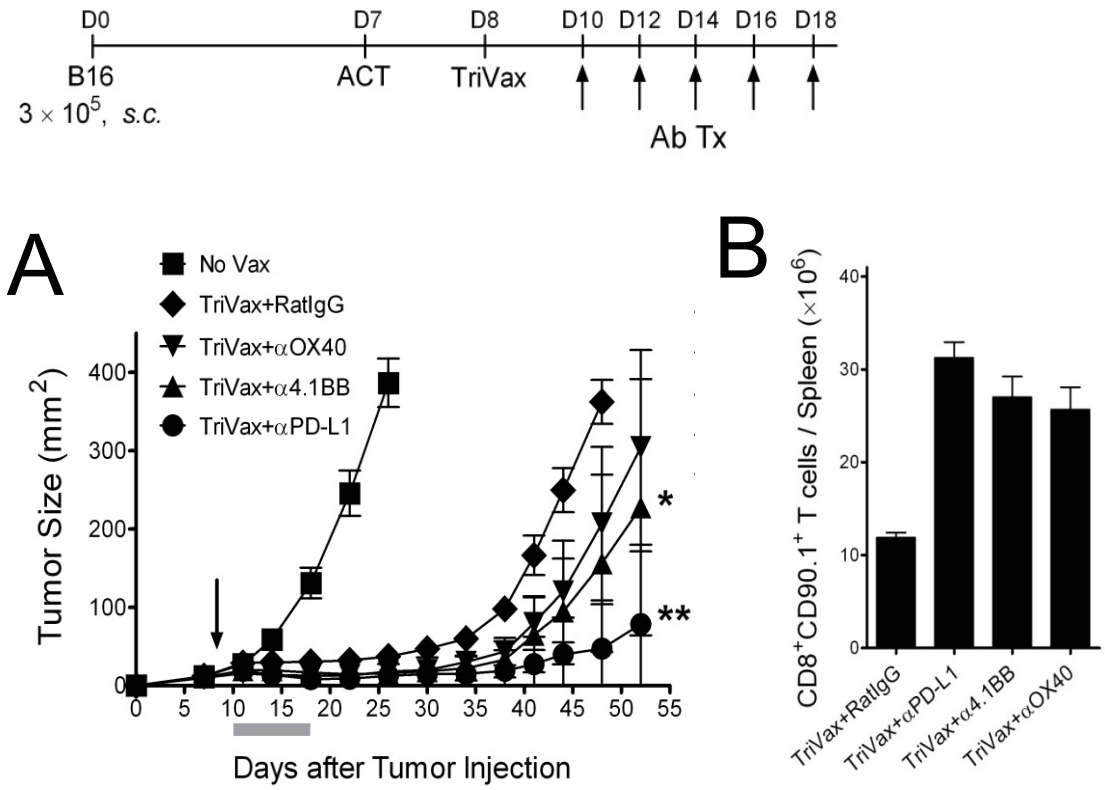
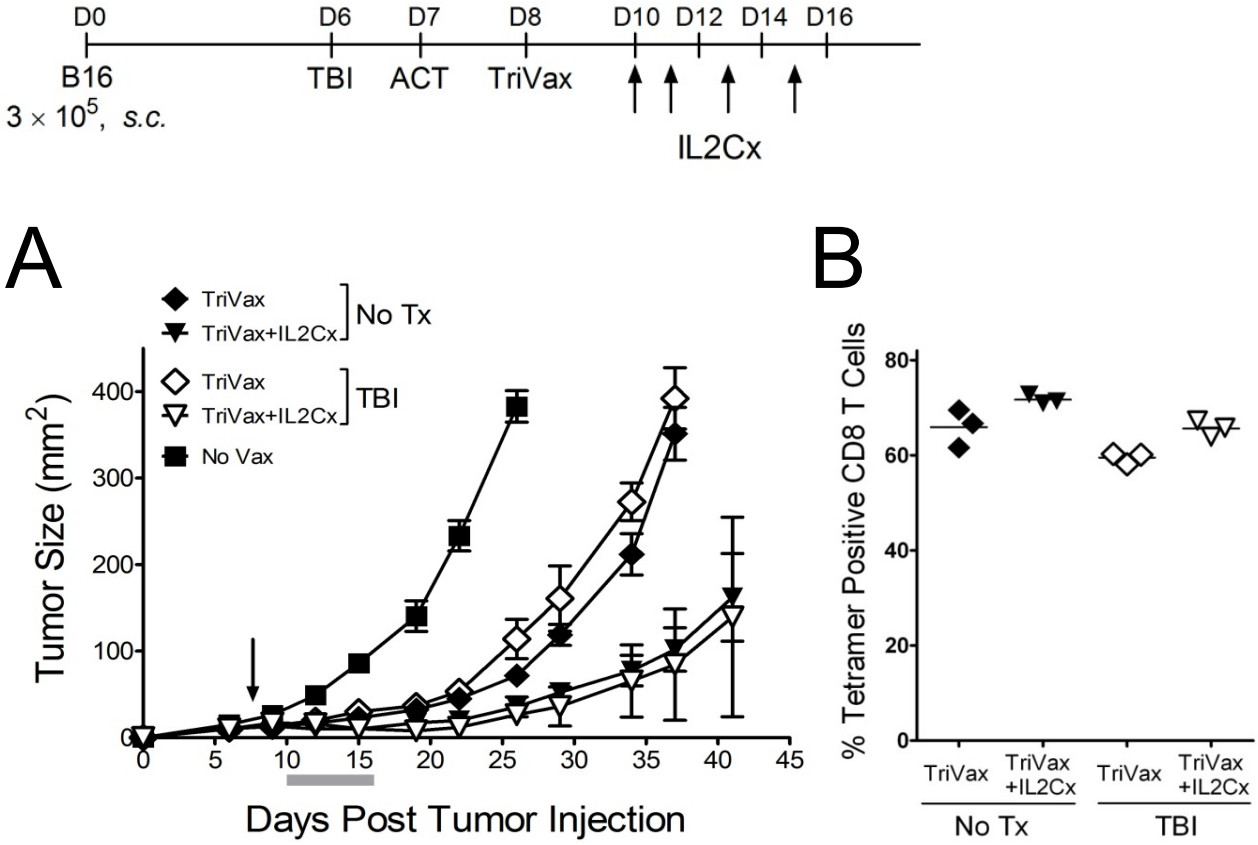


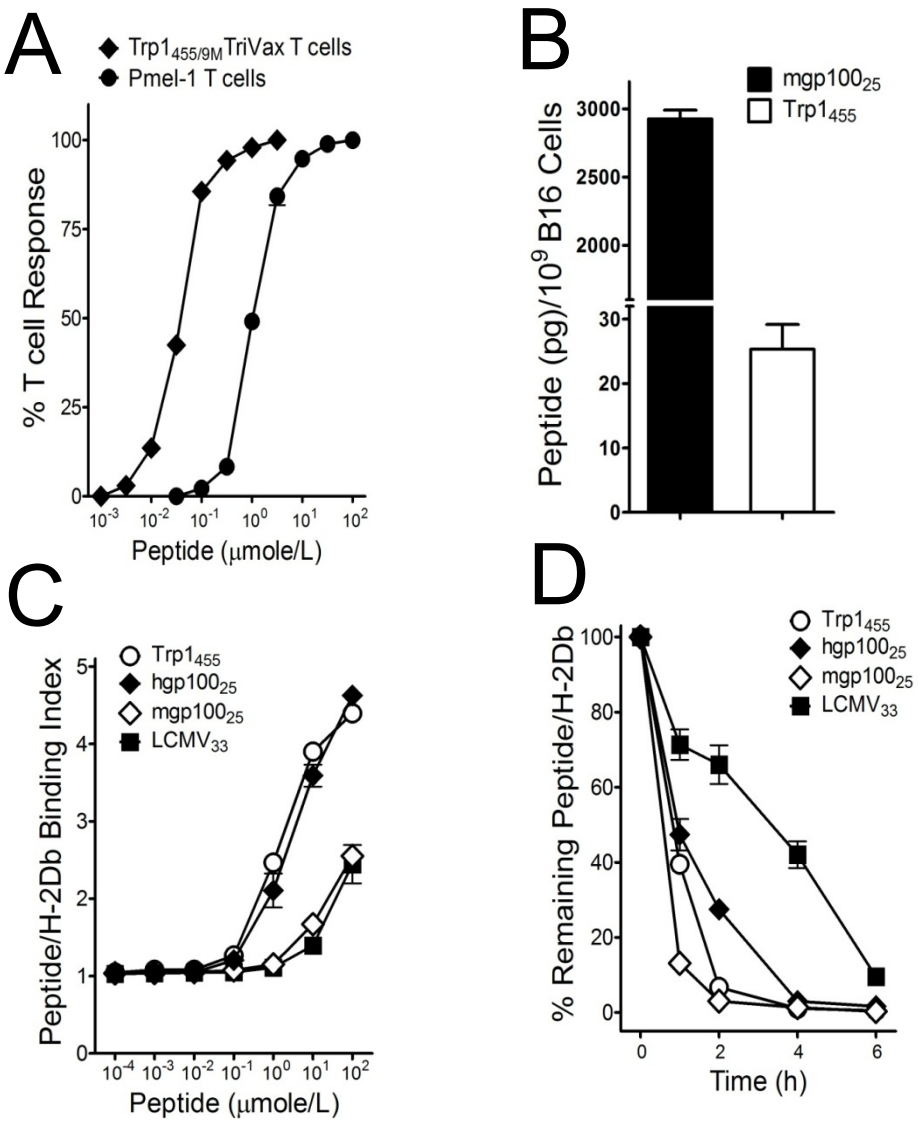
Supplementary Figure S1. **TriVax immunization after ACT expands large numbers of antigen-specific CD8 T cells.** Tumor-free B6 mice (3/group) were adoptively transferred with 3 X 10^5 Pmel-1 cells followed by immunization as indicated. (A) On day 14 after immunization the presence of antigen-specific CD8 T cells in peripheral blood and splenocytes was measured by tetramer staining gating on CD8 positive cells. *Numbers* in each rectangular gate represent the % positive cells of all CD8 T cells. (B) The total numbers of adoptively transferred Pmel-1 CD8 T cells (CD90.1) in spleens were also estimated by flow cytometry. *P* value was calculated with unpaired Student *t* tests comparing with the peptide plus poly-IC group. (C) After TriVax immunization, antigen-specific CD8 T cells were evaluated by tetramer analysis on indicated days using blood samples. *Points*, values from each individual mouse; *horizontal line* represents the average value of the group.



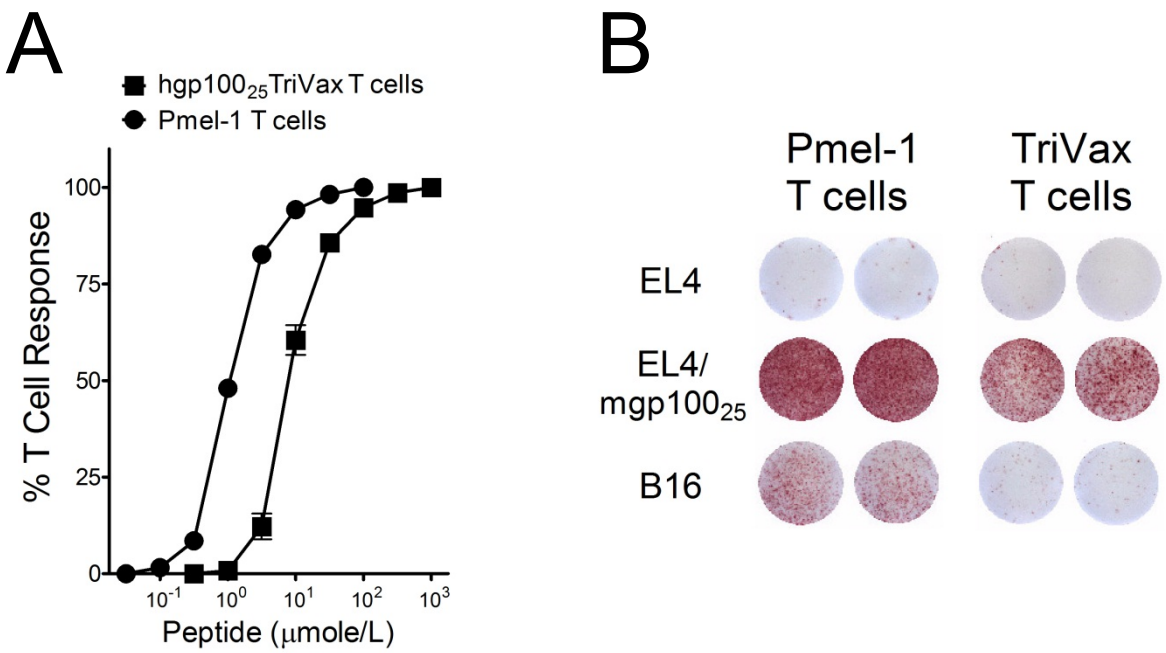
Supplementary Figure S2. **Effects of OX40 and 4-1BB T cell costimulation in the therapeutic efficacy of ACT/TriVax.** (A) B6 mice (4 /group) were inoculated *s.c.* with 3×10^5 B16 cells, and 1 week later received ACT with 3×10^5 Pmel-1 T cells followed a day later by TriVax. Anti-OX40, anti-4.1BB, or anti-PD-L1 monoclonal antibodies were administered *i.p.* 2, 4, 6, 8 and 10 days after TriVax (200 μg IgG/dose. Mice receiving normal rat IgG (RatIgG) and non-vaccinated mice (No Vax) were included as controls. *Points*, mean tumor size for each group; *bars*, SD. Mice with complete tumor rejections: ∗ = 1/4; ∗∗ = 2/4. (B) In a parallel experiment the actual numbers of adoptively transferred CD8 T cells in spleens were enumerated.



Supplementary Figure S3. **Lymphodepletion does not increase efficacy of ACT/TriVax.** (A) B6 mice (4/group) were inoculated *s.c.* on day 0 with 3×10^5 B16 cells, and adoptively transferred on day 7 with 3×10^6 $Trp1_{455}$-specific CD8 T cells into either sublethally irradiated (5 Gy) or normal mice followed by TriVax immunization on day 8 (arrow). Some mice also received IL2Cx as indicated. Non-vaccinated mice (No Vax) were included as controls. *Points*, mean for each group; *bars*, SD. (B) On day 22, blood samples were evaluated for $Trp1_{455}$-specific CD8 T cells by tetramer analysis. *Points*, the value for each individual mouse; *horizontal line* represents the average value of the group.



***Supplementary Figure S4*. T cell epitope factors that determine the effectiveness of ACT.** (A). Antigen-dose responses of purified CD8 T cells isolated from Pmel-1 ACT/TriVax or $Trp1_{455/9M}$TriVax-immunized using RMA-S cells as antigen-presenting cells. Peptides used for the dose-curve responses were $mgp100_{25}$ and $Trp1_{455}$. Results represent the percent maximal T cell responses. (B) The amount of peptide epitopes obtained after mild-acid elution treatment of B16 cells was assessed by a combination of RP-HPLC fractionation and quantitative T cell assays as described in "Methods". (C) Peptide/MHC stabilization assays. Different concentrations of peptides were tested for their capacity to increase H-2Db expression in RMA-S cells measured by flow cytometry. The "Peptide/H-2Db Binding Index was calculated as the ratio of the mean fluorescence intensity (MFI) with peptide / MFI without peptide. (D) Peptide/MHC dissociation assays. After incubating RMA-S cells with peptides for 1 h at 20 μg/ml, the cells were washed and expression of H-2Db was measured at various time points. Results are presented as a percentage of the MFI remaining as compared to 0 h (100%). Peptide $LCMV_{33}$ (KAVYNFATM) was used as a positive H-2Db binder. *Points*, mean for two separate experiments; *bars*, SD.



Supplemental Figure S5. **Comparison of antigen avidity of Pmel-1 cells with the hgp100$_{25}$ TriVax generated endogenous CD8 T cells.** (A) Peptide dose curve responses of TriVax activated Pmel-1 cells and CD8 T cells isolated from B6 mice hgp100$_{35}$ TriVax immunized mice (prime-boost, 16 days apart). Serial peptide dilutions were incubated with RMA-S cells overnight and CD8 T cells were added for another 40 h before harvesting supernatants to measure IFNγ production by ELISA. Results represent the percent T cell response compared to the maximal response (100%) for each T cell tine with SD (error bars) from triplicate cultures. (B) EliSpot assays performed with purified CD8 T cells (same as in panel A) were evaluated for antigen-induced IFNγ secretion by EliSpot against B16 cells and peptide (mgp100$_{25}$) pulsed and un-pulsed EL4 cells. Photos represent examples of wells obtained using 1×10^5 tetramer positive CD8 T cells and 1×10^5 tumor cells per well.