Supporting Information

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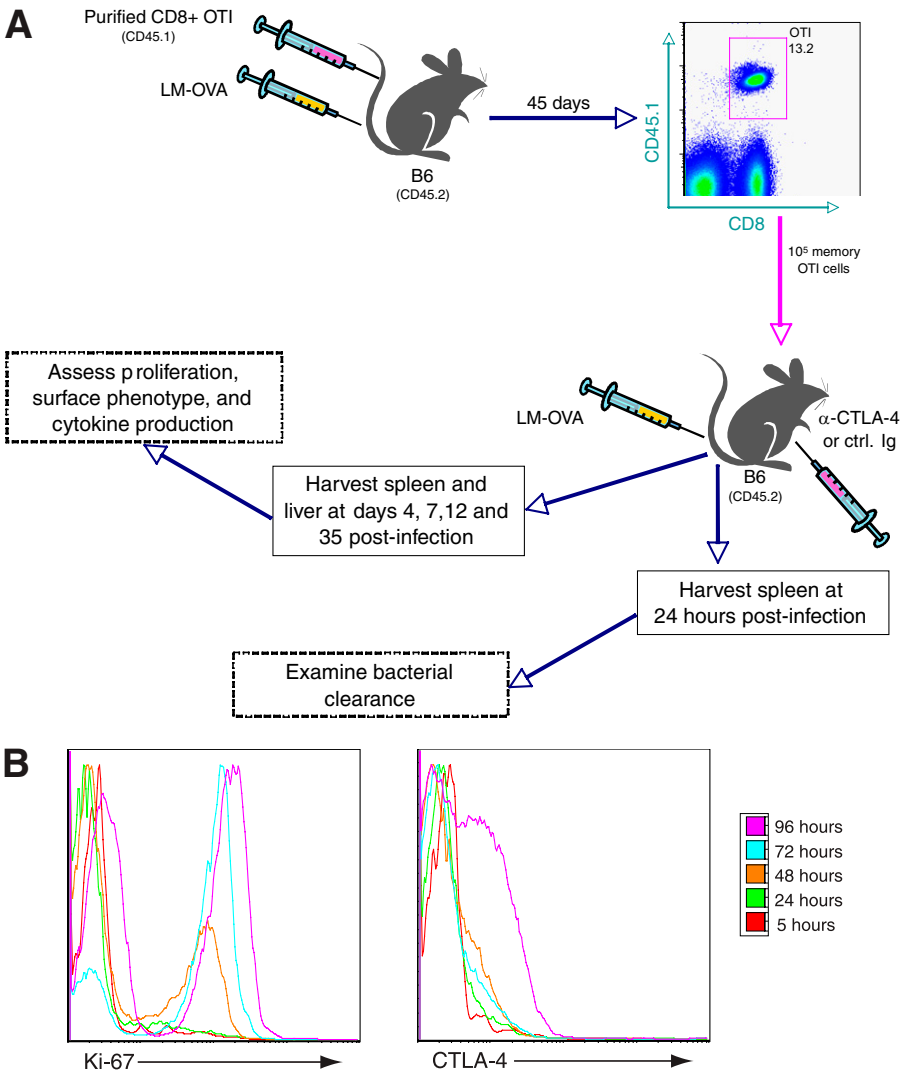

Fig. S1. Generation of antigen-specific memory CD8$^+$ T cells. (*A*) OTI T cell receptor (TCR) transgenic CD8$^+$ T cells on a B6.SJL background (expressing the congenic marker CD45.1) were adoptively transferred into wild-type B6 recipients with a diverse repertoire of T cells bearing the CD45.2 congenic marker. Recipients were then infected i.v. with 5×10^3 cfu *Listeria monocytogenes* that express OVA (LM-OVA) and rested for ≥45 d to allow memory differentiation. Memory OTI.SJL cells were electronically sorted by FACS according to CD8α, CD45.1, and high CD44 expression and adoptively transferred into new naïve B6 recipients (10^5 memory OTI cells per mouse). Recipients were injected i.p. with either blocking anti–CTLA-4 antibody or control hamster Ig and challenged i.v. with 10^5 cfu LM-OVA. Organs from infected mice were harvested after cardiac perfusion with PBS at days 4, 7, 12, and 35 after infection for analysis of memory OTI frequency and cytokine production or at 24 h after infection for evaluation of bacterial burden. (*B*) Splenocytes from memory OTI adoptive transfer recipients were harvested at 5, 24, 48, 72, and 96 h after infection in the absence of CTLA-4 blockade to assess expression levels of CTLA-4 and the proliferation marker K_i-67. Expression levels shown are for memory OTI cells gated as CD8$^+$CD45.1$^+$ and analyzed by flow cytometry.

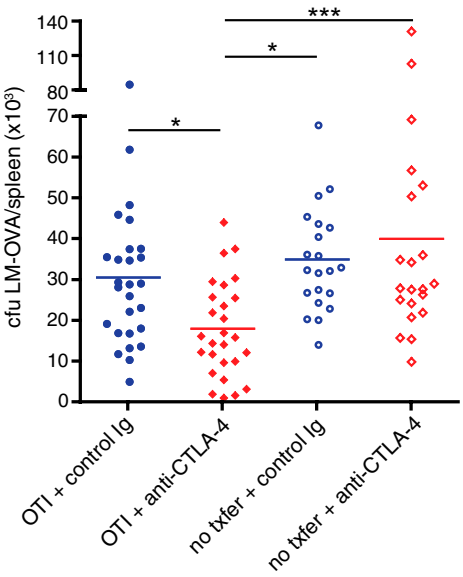

Fig. S2. Blockade of CTLA-4 during memory responses enhances protective immunity. Memory OTI cells generated in vivo were sorted and adoptively transferred into new naïve recipients. Recipient mice then received either control Ig or anti–CTLA-4 and an i.v. infection with a strain of *Listeria monocytogenes* engineered to express ovalbumin (LM-OVA). Bacterial burden is shown from spleens at 24 h after infection in mice that received control Ig or anti–CTLA-4 with or without an adoptive transfer of 10^5 memory OTI cells. Pooled data from three independent experiments with 7–10 mice per group are shown, analyzed using one-way ANOVA. $*P < 0.05$; $***P < 0.001$.

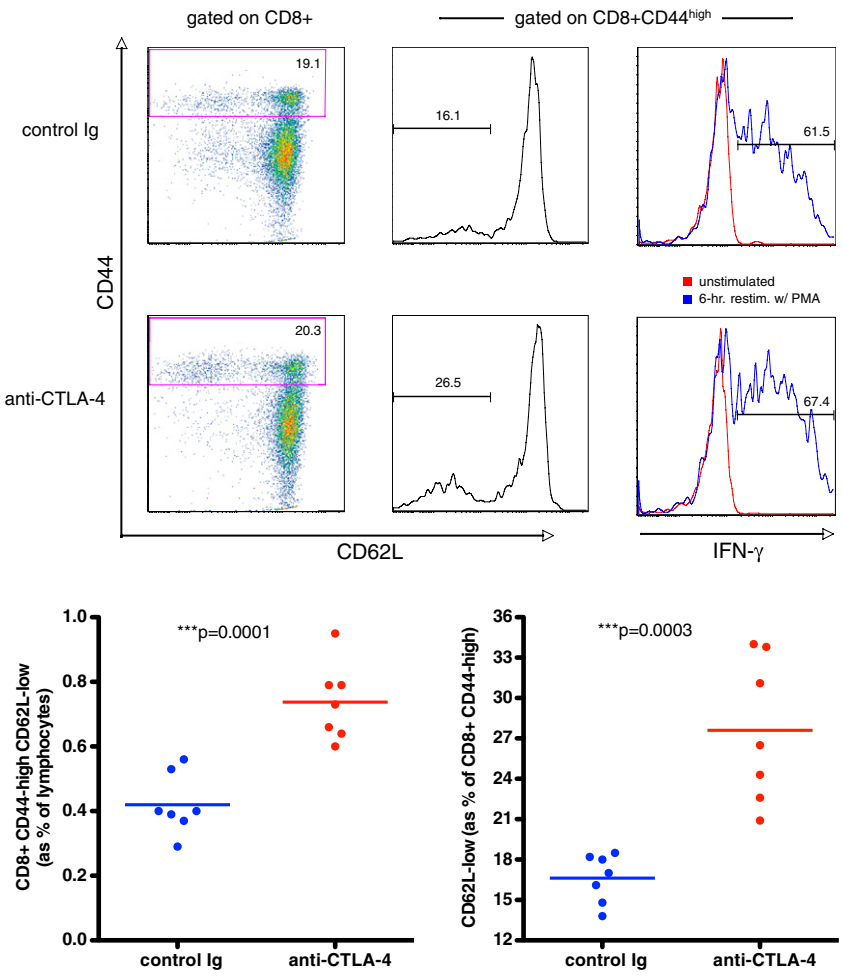

Fig. S3. Endogenous polyclonal CD8$^+$ T-cell memory in mice that received anti–CTLA-4 during priming. Wild-type B6 mice without any adoptive transfer of additional antigen-specific cells received anti–CTLA-4 antibodies or control Ig just before primary infection with a strain of *Listeria monocytogenes* engineered to express ovalbumin. At least 45 d after infection, splenocytes from these mice were analyzed for surface markers and intracellular cytokine production by flow cytometry. Data shown are representative of two independent experiments with five to seven mice per group.

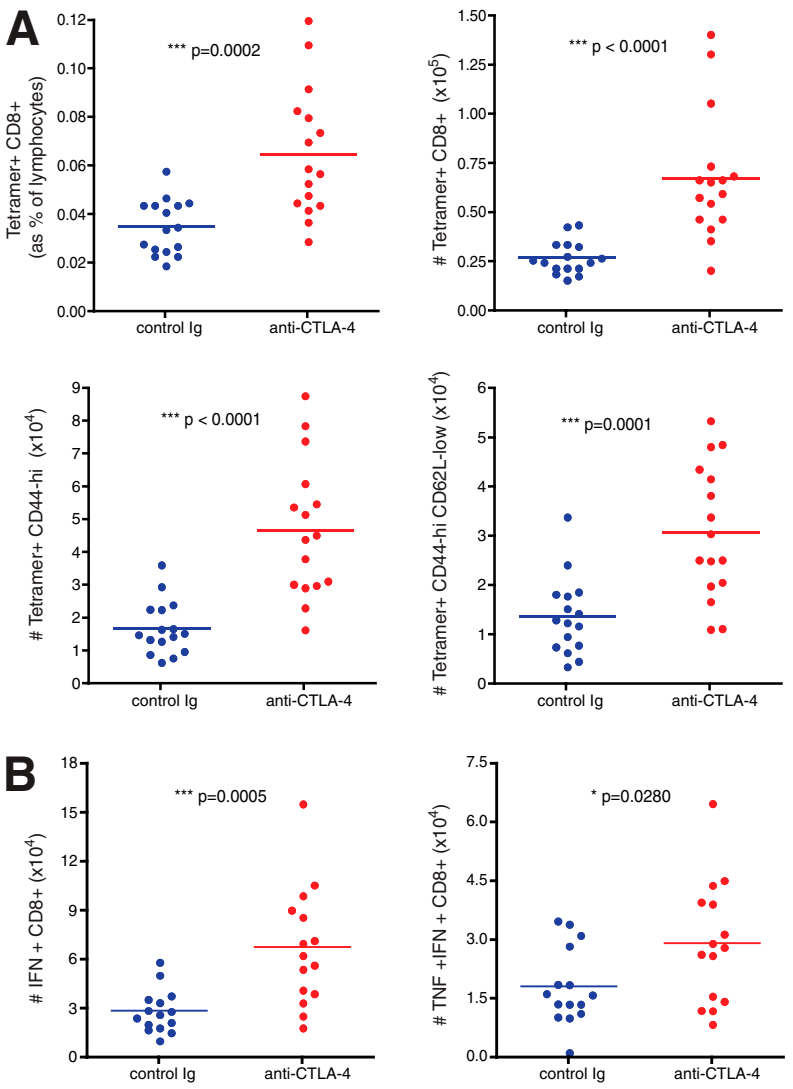

Fig. S4. Blockade of CTLA-4 during priming increases endogenous memory CD8$^+$ T-cell frequency and function. Naïve, wild-type C57BL/6 mice were injected with either control Ig or anti–CTLA-4 just before infection with a strain of *Listeria monocytogenes* engineered to express ovalbumin. Spleens were analyzed at least 45 d after infection, with no further treatment or antigen exposure. (*A*) Flow cytometry using SIINFEKL tetramer was used to quantify frequency of antigen-specific CD8$^+$ memory-phenotype T cells in spleens of mice that received either control Ig or anti–CTLA-4 during priming. Absolute numbers were calculated using lymphocyte cell counts measured with a Guava cell counter. (*B*) Splenocytes were restimulated with SIINFEKL peptide for 5 h and examined by intracellular cytokine staining for antigen-specific production of effector cytokines. Pooled data from three independent experiments are shown with five to six mice per group. Statistical significance was determined with *t* tests.

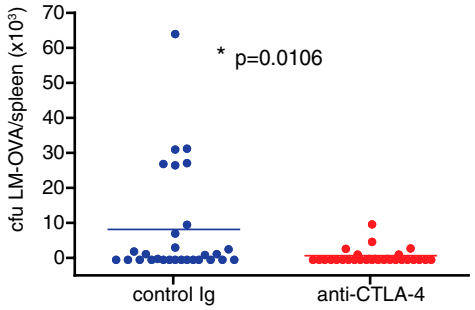

Fig. S5. Blockade of CTLA-4 during priming enhances memory CD8$^+$ T-cell protective immunity. Naïve OTI cells were adoptively transferred into B6 recipients and injected with either control Ig or anti–CTLA-4 just before infection with a strain of *Listeria monocytogenes* engineered to express ovalbumin (LM-OVA). After at least 60 d, mice received a rechallenge infection with LM-OVA. Bacterial burden in spleens at 24 h after infection in mice that received control Ig or anti–CTLA-4 during priming. Data shown are pooled from three independent experiments with 9 to 10 mice per group. Statistical significance was determined with *t* tests.

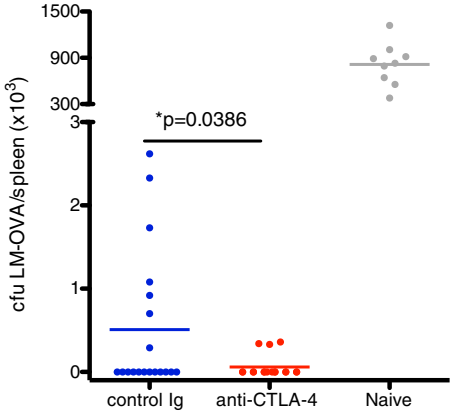

Fig. S6. Enhanced endogenous memory CD8$^+$ T-cell–mediated bacterial clearance in mice that receive CTLA-4 blockade during primary infection. Wild-type B6 mice that received control Ig or anti–CTLA-4 before primary infection with a strain of *Listeria monocytogenes* engineered to express ovalbumin (LM-OVA) were reinfected with a high titer of LM-OVA at least 45 d after priming. At 24 h after infection, bacterial burden was assessed in spleens of infected mice. Data shown are pooled from two independent experiments. n = 10 mice for control Ig and anti–CTLA-4 groups and n = 4 to 5 mice for naïve controls for each experiment. Statistical significance between treatment groups was determined with *t* tests.

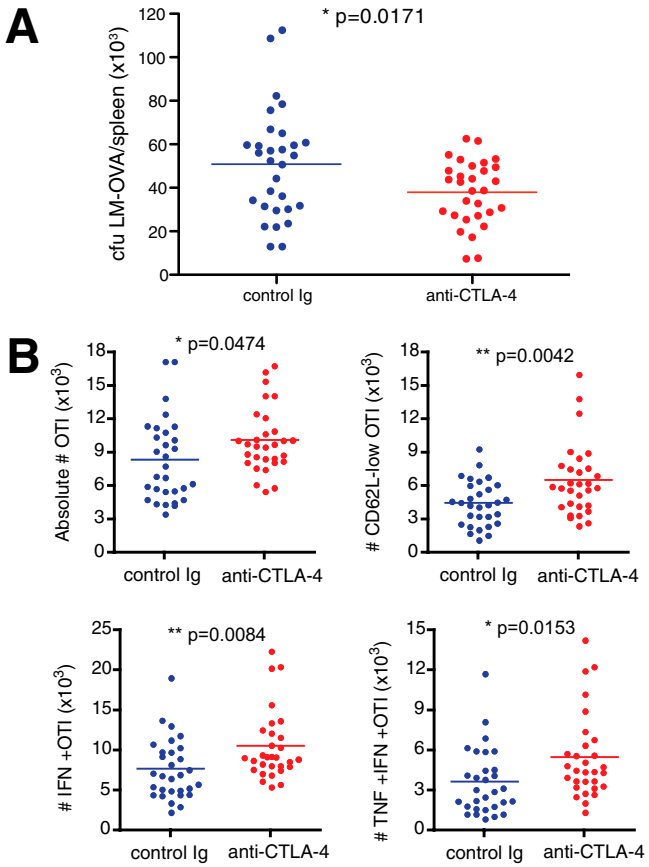

Fig. S7. Blockade of CTLA-4 during priming enhances per-cell memory CD8$^+$ T-cell effector function and protective immunity. OTI cells were adoptively transferred to congenic recipients and injected with either control Ig or anti–CTLA-4 just before infection with a strain of *Listeria monocytogenes* engineered to express ovalbumin (LM-OVA). At least 60 d after primary infection, memory OTI cells from mice that received control Ig or anti–CTLA-4 during priming were sorted by FACS and adoptively transferred into new naïve recipients 18 h before infection with LM-OVA. (*A*) Bacterial burden in spleens at 24 h after infection in mice that received 10^5 transferred memory OTI cells from either control Ig- or anti–CTLA-4–treated mice. (*B*) Quantified absolute number and functional phenotype of transferred memory OTI cells from spleens at 24 h after infection. Data shown are pooled from three independent experiments with 9 to 10 mice per group. Statistical significance was determined with *t* tests.

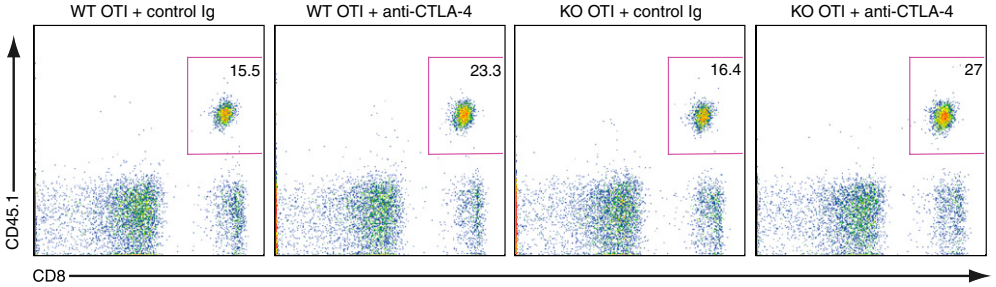

Fig. S8. Anti–CTLA-4 enhancement of memory CD8$^+$ T cells requires cell-extrinsic blockade of CTLA-4. Naïve OTI cells from either wild-type (WT) or CTLA-4–deficient (KO) mice were adoptively transferred into new naïve WT recipients. Recipient mice then received either control Ig or anti–CTLA-4 and an i.v. infection with a strain of *Listeria monocytogenes* engineered to express ovalbumin. Frequencies of antigen-specific OTI cells collected from peripheral blood are shown for day 7 after infection. Each plot is from one mouse (median for each group) of six. Data shown are representative of three independent experiments.