Supporting Information

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SI Methods

Anti–CD127-PE, anti-CD4, and anti-CD8 APC-Cy7 and streptavidin PeCy7 were purchased from BD. Anti–B220-Pacific blue, anti–F4/80-Pacific blue, anti-CD44 PerCP-Cy5.5, and anti-CCR7 biotin were purchased from eBioscience. Anti-CD44 (IM3) Alexa Fluor 488, anti-CD122 (TMβ1.4) Alexa Fluor 488, anti-class MHC II (Y3P) Alexa Fluor 405, and anti–IFN-γ (XMG1.2) Alexa Fluor 647 were produced in the laboratory at National Jewish Health using Alexa Fluor 488, 405, and 657 protein conjugation kits (Molecular Probes). Anti-BrdU Alexa Fluor 488 and Alexa Fluor 647 were obtained from Molecular Probes.

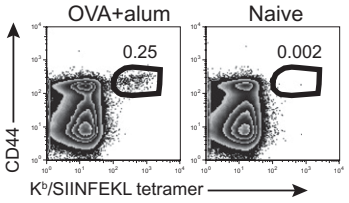

Fig. S1. Immunization with alum and OVA primes antigen-specific CD8 T cells that can be detected by MHC class I tetramers. B6 mice were immunized with OVA plus alum or not immunized, and the percentage of K^b/SIINFEKL tet^+ cells in the spleen was examined 9 d later. The numbers are the percentage of cells within the indicated gate. Cells are gated on $CD8^+CD4^-$ $B220^-F4/80^-MHCII^-$ cells. The data shown are representative of eight experiments with three or four mice per group.

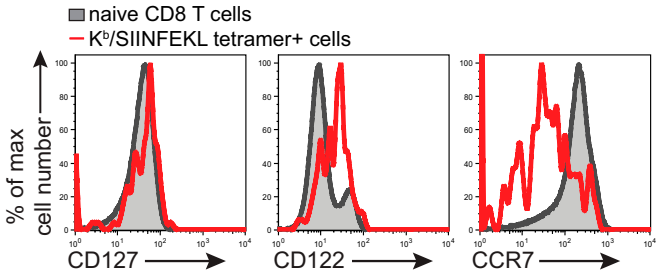

Fig. S2. Memory cells generated with OVA plus alum prime effector memory CD8 T cells. The phenotypes of memory $CD44^{hi}$ K^b/SIINFEKL tet^+ gates (red line) or $CD8^+$ cells (closed gray histogram) were examined at 60 d after immunization with OVA plus alum. The data are representative of three or four experiments with three or four mice per group.

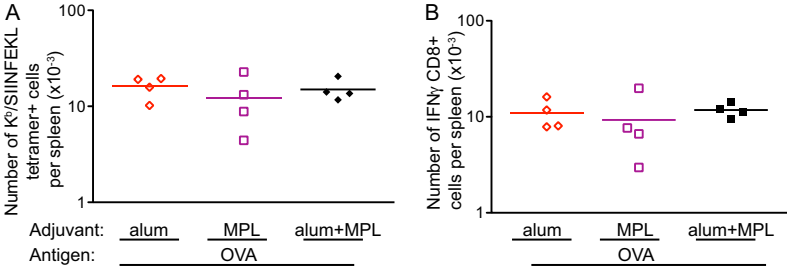

Fig. S3. The addition of MPL affects neither the number of T cells nor the number of IFN-γ–producing cells primed with alum and antigen. (*A*) The number of K^b/SIINFEKL tet^+ CD8 T cells in the spleens of B6 mice primed with OVA with alum, MPL, or both adjuvants was examined at 8 d after immunization. (*B*) Alternatively, splenocytes from these mice were activated ex vivo with SIINFEKL in the presence of Golgi plug for 6 h. The numbers of $CD8^+CD4^-B220^-MHCII^-$ cells that were IFN-γ^+ were examined by intracellular staining. Each symbol represents a mouse, and the line indicates the group mean value. The data are representative of three experiments with four mice per group in each experiment.

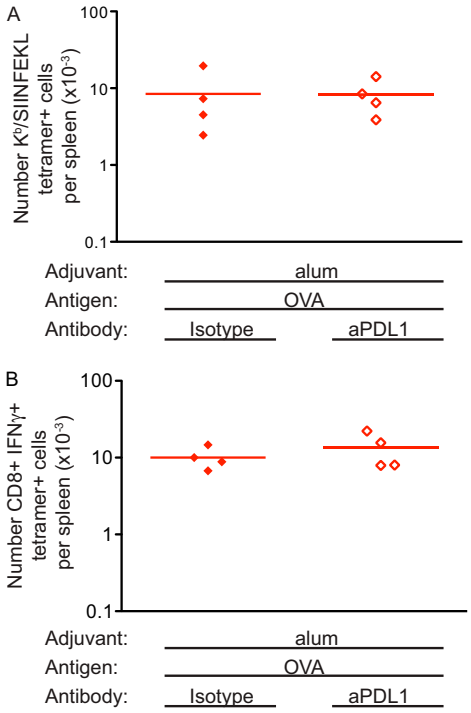

Fig. S4. Blockade of PDL1 affects neither the number of T cells primed nor their IFN-γ response after immunization with alum and antigen. B6 mice were primed with OVA and alum i.p. on day 0 and treated with anti-PDL1 or an isotype control antibody on days 0, 3, and 7. (*A*) The number of K^b/SIINFEKL tet^+ cells present in the spleen was examined 8 d later. (*B*) Alternatively, splenocytes from these mice were activated ex vivo with SIINFEKL in the presence of Golgi plug for 6 h. The numbers of $CD8^+CD4^-B220^-MHCII^-$ cells that were IFN-γ^+ were examined by intracellular staining. Each point represents a mouse, and the line indicates the group mean value, representative of two experiments.

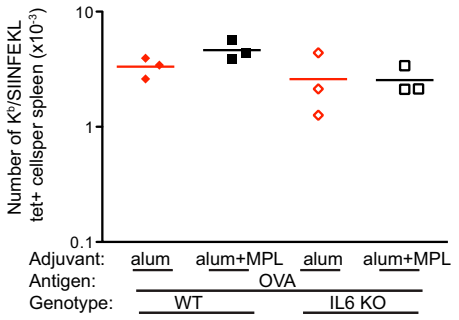

Fig. S5. Immunization with OVA delivered with alum or alum plus MPL primes similar numbers of antigen-specific CD8 T cells in WT and IL-6 KO mice. B6 and IL-6 KO mice were immunized with OVA plus alum or alum plus MPL i.p., and the numbers of K^b/SIINFEKL tet^+ CD8 T cells present in the spleens was examined 8 d later. Each symbol represents a mouse, and the line indicates the group mean. The data are representative of four experiments with three mice per group.

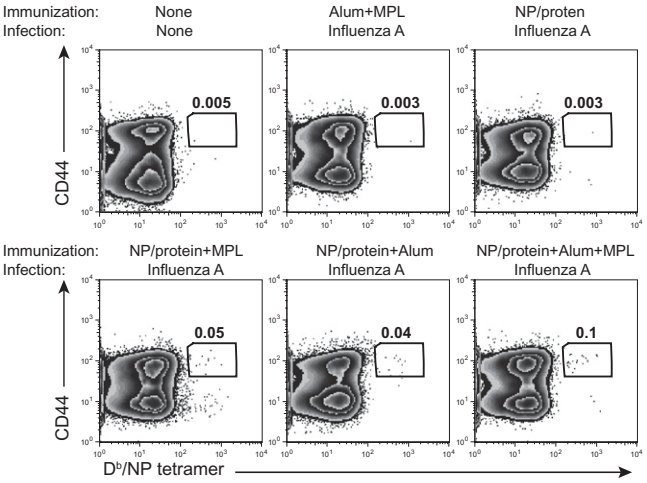

Fig. S6. Antigen-specific memory cells can be found in the lung at early time points after influenza challenge. B6 mice primed with NP/protein alone or with alum, MPL, or alum plus MPL were infected with influenza A intranasally at 95 d after immunization. Four days after infection, one lung lobe was digested with collagenase, and the percentage of CD8 T cells that were D^b/NP tet^+ $CD44^{hi}$ was examined. Cells were gated on live $CD8^+CD4^-F4/80^-B220^-MHCII^-$ lymphocytes. Data are representative of three experiments with four or five mice per group.

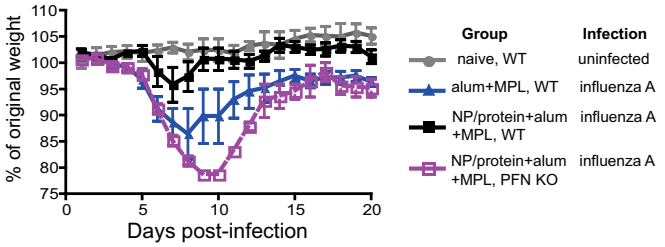

Fig. S7. Memory CD8 T cells must produce a cytotoxic response to provide protection. B6 and perforin KO mice were primed with NP/OVA with alum plus MPL, or B6 mice were given adjuvant alone. Six weeks later, these mice were infected with 150 pfu of influenza A intranasally and were weighed daily. The data are representative of one of two experiments with five mice per group.

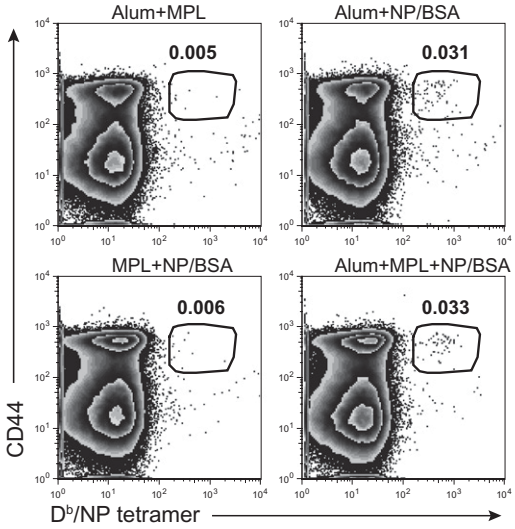

Fig. S8. Similar numbers of memory cells are primed after immunization with antigen delivered with alum or alum plus MPL. B6 mice were immunized with NP/BSA with alum, MPL, or alum plus MPL i.p., or control mice were given alum plus MPL in the absence of antigen. Eight weeks later, the percentages of $CD8^+CD4^-F4/80^-$ $B220^-MHCII^-$ cells that were D^b/NP tet^+ and $CD44^{hi}$ was examined in the spleen. The data are representative of three experiments with four or five mice per group.