Supporting Information

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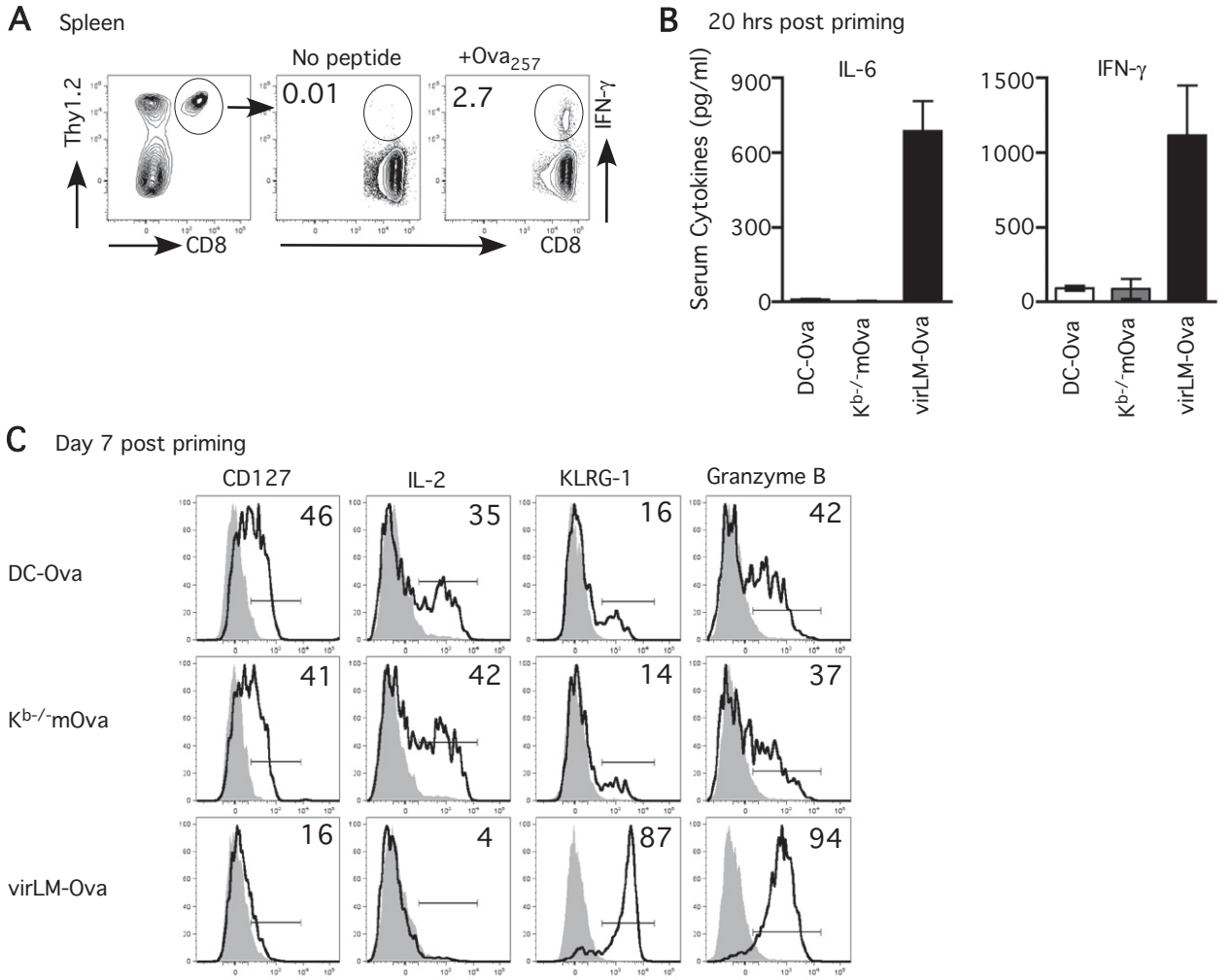

Fig. S1. Cross-priming with cell-associated antigen generates functional antigen-specific CD8 T cells with accelerated memory phenotype in vivo. Naïve C57BL/6 (B6) mice received Ova_{257}-coated dendritic cells (DC) (~10^6 DC/mouse), ~10^7 irradiated $K^{b-/-}$mOva splenocytes, or virulent *Listeria monocytogenes* expressing ovalbumin (virLM-Ova) (~10^5 cfu/mouse). (*A*) Representative dot plots showing detection of Ova_{257}-specific CD8 T cells by ex vivo peptide stimulation from spleen at day 7 after priming from mice receiving irradiated $K^{b-/-}$mOva splenocytes. (*B*) Serum IL-6 and IFN-γ 20 h after immunization. (*C*) Representative histograms showing phenotypic and functional status of Ova_{257}-specific CD8 T cells at day 7 after the three different priming methods indicated. Numbers in histograms represent percentage of cells that are positive for the indicated markers. Shaded histograms represent isotype-control staining. Data are representative of two independent experiments.

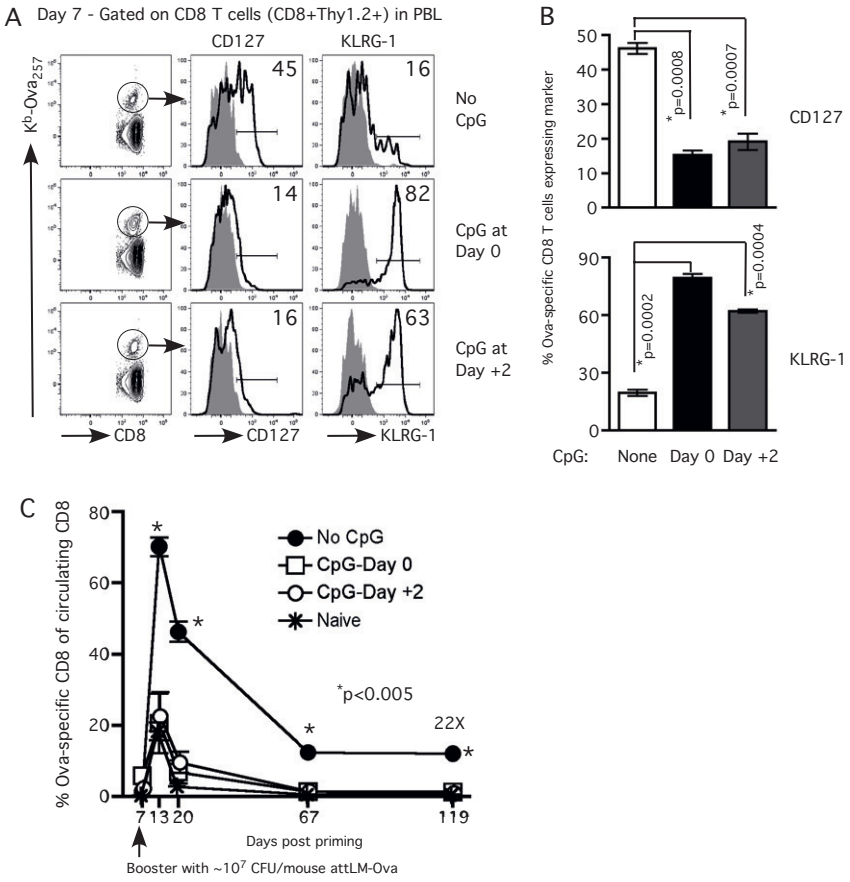

Fig. S2. CpG-induced inflammation prevents accelerated generation of memory CD8 T cells and robust secondary response following cross-priming with cell-associated antigen. Naïve B6 mice received ~10^7 irradiated $K^{b-/-}$mOva splenocytes with or without i.p. coinjection of CpG 1826 (100 μg/mouse) at the indicated time. Mice were boosted with *actA*-deficient *Listeria monocytogenes* expressing Ova (LM-Ova) (~10^7 cfu/mouse) on day 7 after priming. (*A*) Representative dot plots and histograms and (*B*) cumulative data (mean ± SEM, $n = 3$) showing phenotype of Ova_{257}-specific CD8 T cells at day 7 after priming with irradiated $K^{b-/-}$mOva splenocytes. (*C*) Kinetics of Ova_{257}-specific CD8 T-cell response expressed as percentage (mean ± SEM, $n = 3$) of total circulating CD8-positive cells in peripheral blood lymphocytes (PBL) over time after booster immunization at day 7. Data are representative of two independent experiments. Statistical analysis was performed using an unpaired two-tailed *t* test.

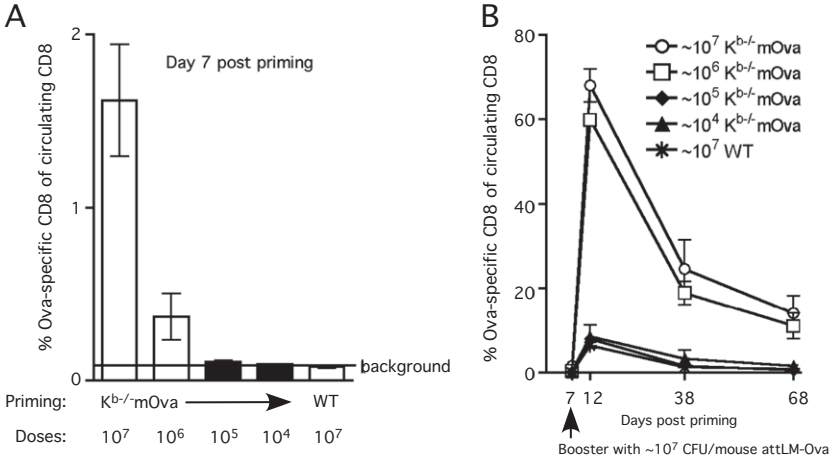

Fig. S3. Dose-dependent antigen-specific CD8 T-cell response following cross-priming with cell-associated antigen and short-interval booster immunization. Naïve B6 mice received different numbers (~10^7, ~10^6, ~10^5, or ~10^4) of irradiated $K^{b-/-}$mOva splenocytes or ~10^7 irradiated WT splenocytes. Mice were boosted with *actA*-deficient LM-Ova (~10^7 cfu/mouse), on day 7 after priming. (*A*) Cumulative data showing frequency of Ova_{257}-specific CD8 T cells expressed as percent (mean ± SEM, $n = 3$ for each group) of total circulating CD8-positive cells in PBL as detected by K^b/Ova_{257} tetramer staining at day 7 after priming. Background indicates nonspecific staining by tetramer K^b/Ova_{257} on PBL from mice immunized with irradiated WT splenocytes. (*B*) Kinetics of Ova_{257}-specific CD8 T-cell response expressed as percentage (mean ± SEM, $n = 3$ for each group) of total circulating CD8-positive cells in PBL over time after booster immunization at day 7 after priming.

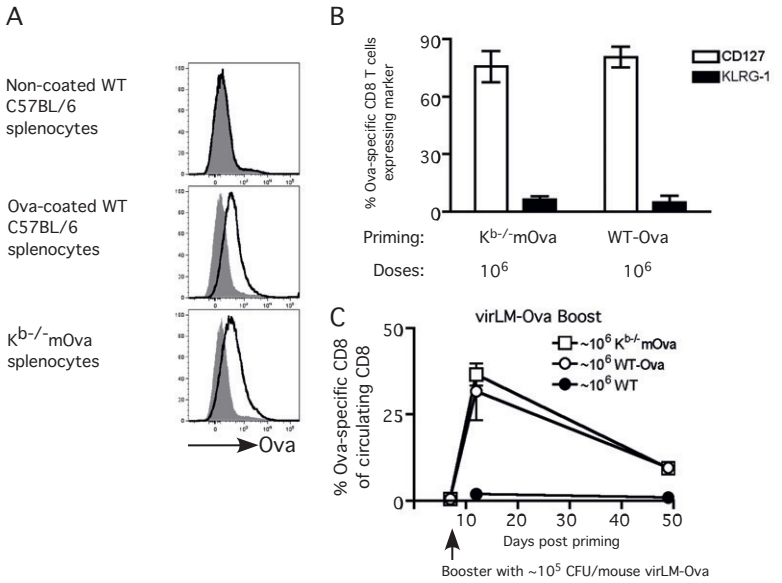

Fig. S4. Irradiated Ova-coated syngeneic splenocytes cross-prime CD8 T cells that can respond vigorously to short-interval booster immunization. Naïve B6 mice were immunized with irradiated WT splenocytes, Ova-coated WT splenocytes, or $K^{b-/-}$mOva splenocytes (~10^6 cells/mouse). Mice were boosted with virLM-Ova (~10^5 cfu/mouse), on day 7 after priming. (*A*) Detection of Ova protein on the surface of irradiated WT splenocytes after incubation with soluble full-length Ova protein. Shaded and unshaded histograms represent Ova-specific staining and isotype control on splenocytes, respectively. (*B*) Cumulative data (mean ± SEM, $n = 3$) showing phenotype of Ova_{257}-specific CD8 T cells at day 7 after priming with either irradiated $K^{b-/-}$mOva splenocytes or Ova-coated WT splenocytes. (C) Kinetics of Ova_{257}-specific CD8 T-cell response expressed as percentage (mean ± SEM, $n = 3$ for each group) of total circulating CD8-positive cells in PBL over time after booster immunization on day 7 after priming with virLM-Ova (~10^5 cfu/mouse). Primary response indicates mice group receiving irradiated, noncoated WT splenocytes and booster immunization 7 d later.

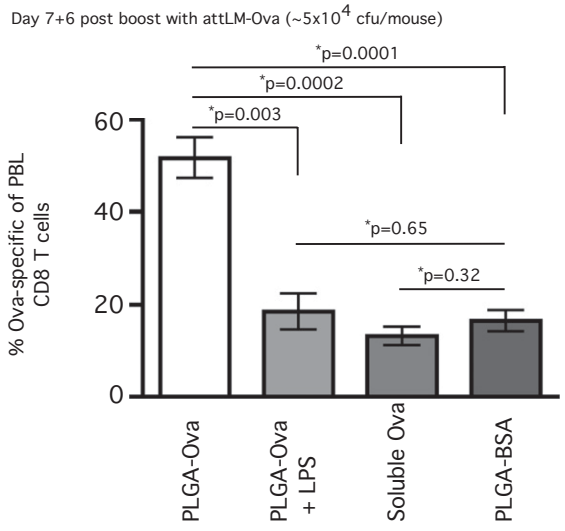

Fig. S5. Robust secondary response following booster immunization is achieved only in mice cross-primed with EndoGrade (LPS free) Ova-coated poly(lactic-coglycolic) acid (PLGA) microspheres but not with soluble Ova or in presence of LPS. Naïve B6 mice were immunized with ~10^9 EndoGrade Ova-adsorbed PLGA microspheres with or without LPS (1 μg), 0.5 μg of soluble OVA, or BSA-adsorbed PLGA microspheres as control and were boosted at day 7 after priming with attenuated *actA*-deficient *Listeria monocytogenes* expressing Ova (attLM-Ova) (~5×10^4 cfu/mouse). The total Ova protein content coated on PLGA microspheres, as determined using the Micro BCA protein assay kit (Thermo Scientific), was ~0.2 μg per mouse. The Ova_{257}-specific CD8 T-cell response was detected by tetramer K^b/Ova_{257} staining and is expressed as percentage (mean ± SEM, $n = 5$ for each group) of total circulating CD8-positive cells in PBL at day 6 after booster immunizations. *Statistical analysis was performed using an unpaired, two-tailed *t* test.

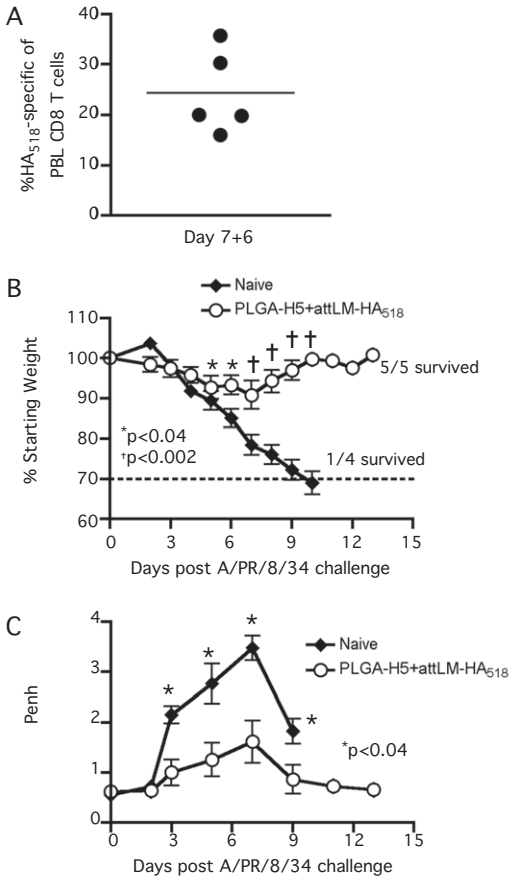

Fig. S6. Rapid generation of effector CD8 T cells and protective heterosubtypic immunity via cross-priming with H5-PLGA microspheres followed by booster immunization at day 7. Naïve BALB/c mice were immunized with ~10^9 PLGA microspheres coated with recombinant HA H5 protein. Mice received booster immunization with *actA*-, IntB-deficient *Listeria monocytogenes* expressing the H-2K^d-restricited influenza epitope, IYSTVASSL (attLM-HA$_{518}$) (~10^7 cfu/mouse) on day 7 after priming. (*A*) Frequency of HA$_{518}$-specific CD8 T cells among circulating PBL CD8 T cells at day 6 after boosting (day 7 + 6). On day 7 after boosting (day 7 + 7), naïve BALB/c mice (n = 4) and cross-prime–boosted mice (n = 5) were challenged with a lethal dose (~5 LD$_{50}$) of influenza A/PR/8/34 (H1N1). (*B*) Morbidity is measured by weight loss and expressed as percent of starting weight. Numbers on the graph indicate the number of surviving mice/total number of mice. (*C*) Airway resistance was measured using a whole-body plethysmograph (Buxco Electronics) and expressed as maximal enhanced pause (Penh) values. Baseline Penh values for each mouse were recorded before and at the indicated time points after challenge with influenza A/PR/8/34. Statistical analysis was performed using an unpaired, two-tailed t test.