Supporting Information

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SI Materials and Methods

Mice. C57BL/6 (B6) mice ($H2^b$; Thy1.1; B6.CD45.1, B6.CD45.2, and B6.GFP) were used as donors of hematopoietic stem cells (HSCs) and lymphocytes for BALB.B hosts ($H2^b$, Thy1.2; CD45.2). HSC donors were 6 to 10 wk of age, and recipients were at least 8 wk old. Mice were bred and maintained at the Stanford University Research Animal Facility. All studies were approved by the Stanford University Administrative Panel on Laboratory Animal Care.

Isolation of Hematopoietic Stem Cells. Bone marrow was flushed from tibiae and femora into HBSS/2% FBS, enriched for c-Kit (3C11) cells by magnetic column separation (CD117 MicroBeads, MACS Separation Columns LS; Miltenyi Biotec), and KTLS-HSCs were purified by FACS-sorting, selecting for c-Kit$^+$ Thy1.1$^{lo\text{-}int}$ Sca-1$^+$ Lin^{neg} cells.

Isolation of T Lymphocytes. For the adoptive transfer of total T cells (ToTCs), CD4 and CD8 T cells were extracted from donor spleens by magnetic column separation (CD4 and CD8α MicroBeads; Miltenyi Biotec). Groups of donors were immunized by infection with a sublethal dose of 1×10^5 pfu lacZ-tagged murine CMV (MCMV) RM427$^+$ 4 and 2 wk before hematopoietic cell transplantation (HCT). CD62L$^+$CD44$^-$ naive, CD62L$^{+/-}$CD44$^+$ memory, and M45-tetramer$^+$ CD8$^+$ cells, were FACS-separated from enriched splenic CD4$^+$ and CD8$^+$ populations from immunized donors.

HSC Transplantation. BALB.B mice were lethally irradiated with 800 cGy (two split doses >3 h apart), and 3,000 KTLS-HSCs were infused via tail vein within 3 h after the second radiation. In cotransfer experiments, 2.5 to 4×10^6 ToTCs (CD4$^+$+CD8$^+$ = ToTC), 3×10^5 naive, 1×10^5 memory CD8$^+$ T cells, or 6,000 M45-tetramer$^+$ CD8$^+$ cells enriched from splenocytes were injected simultaneously with the HSC. Groups of animals were given daily cyclosporine A (CSA) injections starting on the day of transplantation at a dose of 250 μg (i.e.,10 mg/kg for a 25-g mouse) until harvest.

MCMV Infection. Groups of transplanted mice were challenged intraperitoneally with 1×10^5 (i.e., sublethal) to 5×10^5 (i.e., threshold-lethal) pfu of MCMV RM427$^+$ at 2 to 8 wk post-transplantation. Two weeks postinfection, lymphatic organs were harvested for FACS and enzyme-linked immunosorbent spot (ELISPOT) analysis.

Staining for Flow Cytometry. For FACS-purification of HSC antibodies against c-Kit (2B8), Thy1.1 (OX-7), Sca-1 (D7), and the lineage markers CD3ε (145-2C11 or 17A2), CD4 (GK1.5), CD5 (53-7.3), CD8α (53-6.7), B220 (RA3-6B2), Gr1 (RB6-8C5), Mac1 (M1/70) and TER-119 (TER-119) were used. To identify T-cell subsets, anti-CD62L (MEL-14), and CD44 (IM7) antibodies were used. Phenotype analysis was performed by using B220 (RA3-6B2), Thy1.1 (OX-7), Thy1.2 (53-2.1), CD3ε or TCRβ (H57-597), CD4, CD8 (5H10), NKp46 (29A1.4), RORγt (AFKJS-9), IL-17A (ebio17B7), and IFN-γ (XMG1.2). Donor vs. host were determined by Thy1.1, Thy1.2, CD45.1 (A20) and CD45.2 (104), or GFP. Antibodies were from eBioscience, Biolegend, Invitrogen, or BD Biosciences Pharmingen. M45-tetramers (HGIRNASFI; H-2D^b) were manufactured by the National Institutes of Health tetramer facility.

Tissues were manually processed into single cell suspensions. Mononucleated cells were isolated from perfused livers by density gradient (Ficoll-Paque Plus; GE Helathcare). Cells were blocked with Fcγ-block (10 min), and antibody-stained (30 min; 1 h for tetramers) on ice. Samples were analyzed and sorted on the Stanford shared FACS facility Hi-Dimensional FACS instruments (LSR II; FACSAria with DiVa electronics; Becton Dickinson).

For measurement of IL-17A and IFN-γ, cells were stimulated with phorbol myristate acetate, ionomycin, and monensin for 5 h at 37 °C. Washed cells were incubated with a viability dye for 30 min (LIVE/DEAD Fixable Aqua Dead Cell Stain Kit; Invitrogen), and surface-stained as described earlier. Specimens were fixed and permeabilized by using a Foxp3 staining kit (eBioscience) according to the manufacturer's instructions, and antibody-stained for intracellular cytokines.

IFN-γ ELISPOT Assay. Ninety-six–well PVDF plates coated with anti–IFN-γ antibody (0.5 μg per well; Mabtech) were blocked with RPMI 10% FBS for 2 h before MCMV M45 peptide (HGIRNASFI-COOH; 0.1 ng/100 μL per well; Stanford Protein and Nucleic Acid Facility) was added. A total of 2.5 to 3×10^5 sorted splenocytes (in 50 μL) were plated in duplicate and incubated for 20 h at 37 °C. Plates were incubated with a secondary anti–IFN-γ–biotinylated antibody (1 μg/mL; 2 h) and streptavidin-alkaline phosphatase (1:1,000; 1 h), and developed with BCIP/NBT substrate dye (~20 min until color change; Moss). The reaction was stopped with tap water, and plates were dried overnight before analysis (Zellnet). Counted numbers of spots per well represented individual IFN-γ–secreting cells.

Statistical Methods. Statistical differences and their P values for groups were assessed by a two-tailed Student t test by using Microsoft Excel software and GraphPad Prism 4.0 software. Kaplan–Meier survival curves and column-bar diagrams display the mean and SEM for each group, and were created using GraphPad Prism 4.0 software. Weight curves were created by using Excel software.

Histopathology. Fragments of the liver and intestine, lymph nodes, and thymuses from representative animals were fixed in 10% neutralized formalin, dehydrated in graded ethanol, and embedded in paraffin. Paraffin embedding and H&E staining was performed by the Histo-Tec Laboratory. Pictures were taken on a Leica CTR 5000 digital microscope with a Leica FDC 425 camera.

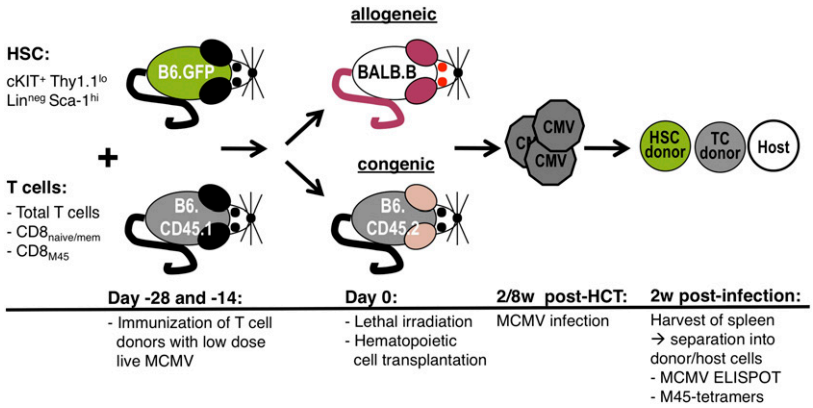

Fig. S1. Schema of experimental design. Congenic strains of C57BL/6 (B6) mice were used as donors of cKit^{+}Thy1.1^{lo}Sca-1^{+}Lin− HSCs (GFP.B6) and ToTCs or T-cell subsets (CD45.1 B6). For some experiments, CD45.1 B6 T-cell donors were immunized with live MCMV virus. On day 0, the day of transplantation, allogeneic BALB.B (CD45.2) mice and congenic CD45.2 B6 mice were lethally irradiated and infused with HSCs alone or supplemented with ToTCs or T-cell subsets. At 2 or 8 wk posttransplantation, recipient mice were infected with live MCMV virus. At 2 wk postinfection, mice were killed and lymphatic organs were used for FACS analysis and ELISPOT assays.

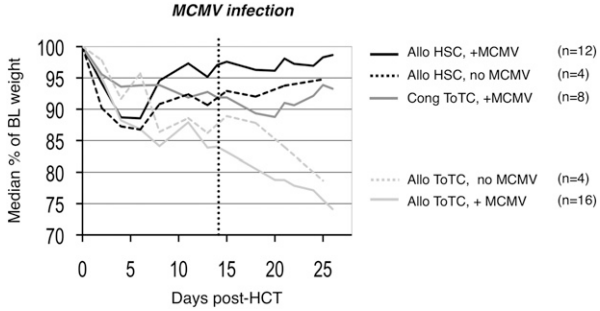

Fig. S2. Weight course of BALB.B or B6 mice transplanted with B6 HSCs or HSC+ToTCs and infected at 2 wk after HCT with a sublethal dose of 1×10^5 pfu MCMV RM427^{+}. Weight curves displaying median percent of baseline (BL) weight show severe weight loss in allogeneic HSC+ToTC recipients with or without MCMV infection, and prompt recovery in HSC and congenic HSC+ToTC recipients with or without MCMV infection.

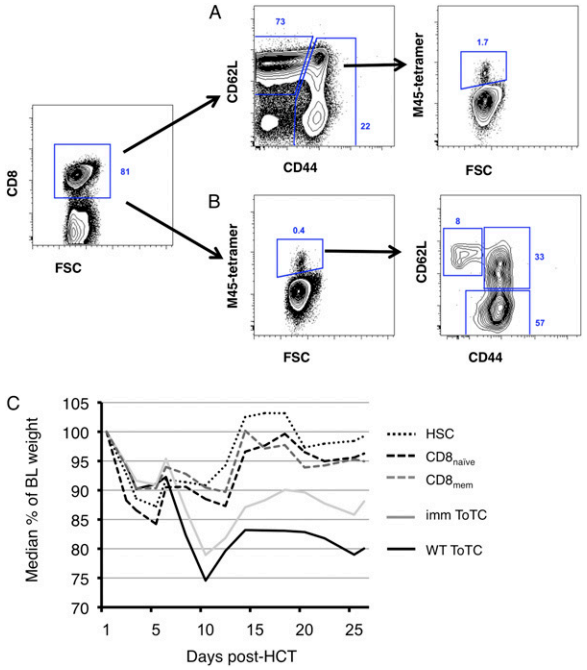

Fig. S3. FACS-sorting schema for CD8-enriched splenocytes from B6 donors that were immunized with live MCMV at 4 and 2 wk before transplantation. (*A*) Splenocytes were separated into naive ($CD62L^{+}CD44^{-}$) and memory ($CD62L^{+/-}CD44^{+}$) $CD8^{+}$ cells. Of all memory $CD8^{+}$ cells, approximately 1.7% were M45-tetramer–positive (*Right*). (*B*) Of all $CD8^{+}$ cells, 0.4% were positive for the M45-tetramer. The majority of those MCMV-specific $CD8^{+}$ cells had an effector memory ($CD62L^{-}CD44^{+}$) or central memory ($CD62L^{+}CD44^{+}$) phenotype. (*C*) Weight curves for BALB.B recipients showed that both recipients of HSC+ToTC$_{WT}$ (donor MCMV-naive) and HSC+ToTC$_{imm}$ (donor MCMV-immunized) had graft-vs.-host disease (GVHD)-related weight loss. Recipients of HSCs alone, HSC+CD8$_{naive}$, or HSC+CD8$_{mem}$ cells had no overt signs of GVHD.

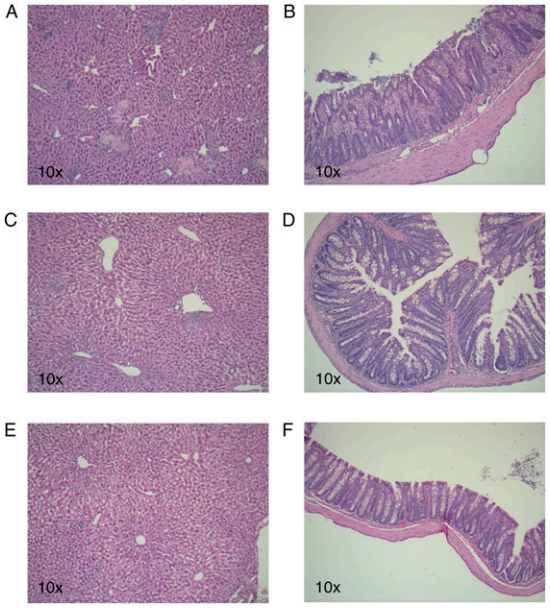

Fig. S4. H&E staining of livers (*Left*) and intestines (*Right*) of representative BALB.B recipients of B6 HSC+ToTCs (*Top*), HSC+CD8$_{naive}$ cells (*Middle*), and HSC+CD8$_{mem}$ cells (*Bottom*). Livers (*A*) and intestines (*B*) of HSC+ToTC recipients showed typical signs of acute GVHD, as indicated by cellular infiltrates and necrotic foci with disruption of the tissue morphology. (*C–F*) No signs of GVHD were evident in liver and intestine of recipients of HSC+CD8$_{naive}$ (*C* and *D*) or HSC+CD8$_{mem}$ cells (*E* and *F*).

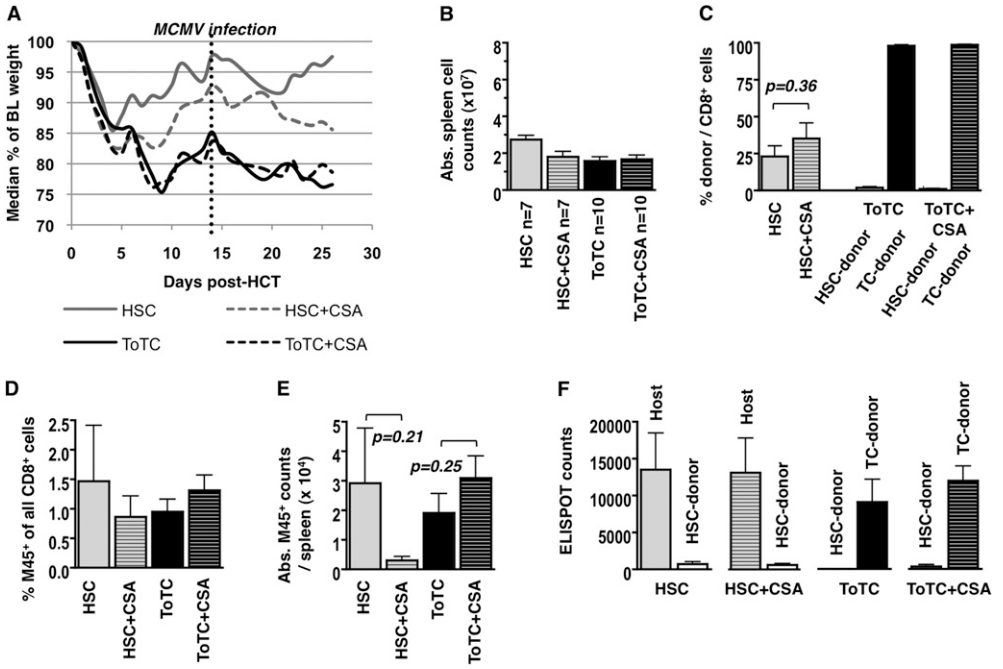

Fig. S5. Assessment of CSA on immune reconstitution. Allogeneic HSC vs. HSC+ToTC recipients were treated with CSA and infected with MCMV 2 wk after HCT. (*A*) Weight curves showed that all mice lost weight after infection. HSC recipients that did not receive CSA regained weight, whereas those given CSA continued to lose weight. HSC+ToTC recipients had comparable curves of weight loss, with or without CSA. (*B*) Comparable absolute spleen cell numbers in recipients of HSCs vs. HSC+ToTCs, with or without CSA. (*C*) FACS analysis showed no influence of CSA treatment on $CD8^+$ T-cell chimerism. HSC recipients were mixed chimeras with host ($Thy1.2^+$ $CD45.2^+$) exceeding donor ($Thy1.1^+$ $CD45.2^+$) $CD8^+$ T cells 4 wk post-HCT. In recipients of HSC+ToTCs, $CD8^+$ T cells were derived from cotransferred cells ($Thy1.1^+CD45.1^+$), with few cells originating from the donor HSC ($Thy1.1^+$ $CD45.2^+$) and no measurable residual host cells. (*D* and *E*) FACS analysis of M45-tetramer$^+$ $CD8^+$ T cells in spleens from mice that did or did not receive CSA showed no significant differences with regard to the proportions (*D*) or absolute numbers (*E*) of M45-tetramer$^+$ cells. (*F*) ELISPOT confirmed that the addition of CSA to the treatment did not alter the anti-MCMV reactivity of host or donor cells. In the HSC recipients, residual host cells mounted the strongest anti-MCMV response, exceeding the reactivity of cotransferred allogeneic T cells in HSC+ToTC recipients.

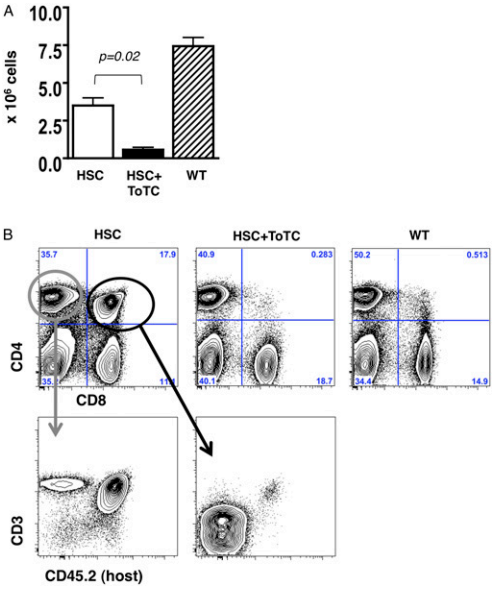

Fig. S6. (*A*) Absolute numbers of mesenteric lymph node cells were higher in recipients of HSCs vs. HSC+ToTCs. (*B*) In HSC recipients, a $CD3^{neg\text{-}low}$, donor-derived ($CD45.2^-$) $CD4^+CD8^+$ double-positive population was detected in mesenteric lymph nodes at 3 wk posttransplantation. Single-positive $CD4^+$ and $CD8^+$ T cells were $CD3^+$ and mainly host-type ($CD45.2^+$). HSC+ToTC recipients and WT controls lacked this $CD4^+CD8^+$ double-positive population. $CD4^+$ cells in HSC+ToTC recipients were derived from mature donor T cells ($CD45.2^-$).

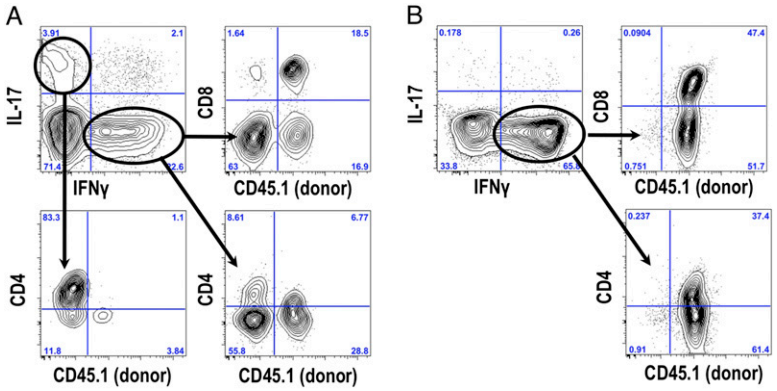

Fig. S7. IL-17A and IFN-γ expression of mononuclear cells in the liver of representative mice given HSCs (*A*) or HSC+ToTCs (*B*). FACS plots display the proportion of IL-17A and IFN-γ cells of all live cells. (*A*) In recipients of HSC, approximately 4% of live cells expressed IL-17A (*Upper Left*), and the source of this cytokine was residual host $CD4^+$ cells (*Lower Left*). IFN-γ was expressed by approximately 20% of all live cells (*Upper Left*) and was partly derived from donor $CD8^+$ ($CD45.1^+$; *Upper Right*) and $CD4^+$ cells (donor and host), whereas the greatest proportion was produced by non–T-cell populations. (*B*) In recipients of HSC+ToTCs, there was no clear expression of IL-17A detectable (*Upper Left*), whereas approximately two thirds of all cells expressed IFN-γ. IFN-γ was secreted by donor $CD8^+$ (*Upper*) and $CD4^+$ (*Lower*) cells.