Supporting Information

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Supplemental Materials and Methods

Mice. Female C57BL/6 and B6-Ly5.2 mice between 5 and 8 weeks of age were purchased from the National Cancer Institute. Female B6.129S4-*Il2ratm1Dw*/J (CD25$^{-/-}$) mice were purchased from Jackson Laboratories or were kindly provided by Dr. Charles Surh (The Scripps Research Institute, La Jolla, CA). Female B6.129S2-*Cd28tm1Mak*/J (CD28$^{-/-}$) mice were purchased from Jackson Laboratories. Female ABBN12-N (class II$^{-/-}$) mice were purchased from Taconic. CD40$^{-/-}$ mice were obtained from Dr. Randy Noelle (Dartmouth College, Hanover, NH). CD80/86$^{-/-}$ mice were obtained from Dr. Lloyd Kasper (Dartmouth College, Hanover, NH). All animal protocols were approved by the Animal Care Committee at University of Connecticut Health Center, Farmington, CT, and the Animal Care and Use Program at Dartmouth College, Hanover, NH.

Generation of Bone Marrow Chimeras. Bone marrow cells were obtained from femurs and tibias of CD25$^{-/-}$ and B6-Ly5.2 mice. B6-Ly5.2 recipient mice were lethally irradiated with approximately 1,000 rads and subsequently injected i.v. with a total of 10^6 bone marrow cells containing a mixture of CD25$^{-/-}$ and B6-Ly5.2 at a 2:1 ratio. Mice were left for at least 50 days before infection.

CD4 Depletion. Mice were given 500 μg of the GK1.5 monoclonal antibody i.p. one day before infection, 500 μg at the time of infection, and 200 μg of GK1.5 every three 3 days for the duration of the experiment. Depletion was confirmed using the RM4-5 mAb.

Infection of Mice. Mice were given primary infections by: i.v. infection with 10^5 PFU of VSV-ova or 10^3 CFU of LM-ova or i.p. infection with 2×10^6 VV-WR. Secondary infections of mice were performed as follows. VV-WR memory mice were infected i.v. with 1×10^6 CFU of attenuated (*ActA*$^-$ *LLO*$^-$) LM expressing the B8R epitope (LM-B8R) provided generously by Dr. Ross Kedl (National Jewish Medical Research Center, University of Colorado, Denver, CO). Both VSV-ova and LM-ova memory mice were challenged with 5×10^4 CFU of LM-ova.

IL-2 Treatment of Mice. Helpless mice were administered 15,000 U of recombinant human IL-2 (National Institutes of Health, Bethesda, MA) i.p. from day 1–4 postinfection, as previously performed (1).

Tissue Sample Preparation and Flow Cytometric Analysis. Mice were either bled at the indicated time points or single cell suspensions of the indicated tissues were prepared by collagenase digestion as previously described (2). The H-2K^b tetramer containing the ovalbumin derived peptide SIINFEKL was generated as previously described (3). The H-2K^b /B8R peptide (TSYKFESV) tetramer was obtained from the National Institutes of Health Tetramer Core Facility, Atlanta, GA. Cell were stained as previously described (4, 5). Samples were analyzed on a BD LSR-II or BD FacsCanto flow cytometer. Data analysis was done using FlowJo software.

Measurement of BrdU Incorporation. Infected mice were i.p. injected twice, 12 h apart, with 1mg BrdU on either day 4 or 8 postinfection. Mice were killed 24 h later and spleen cells were stained with the appropriate cell surface molecules, followed by staining with the anti-BrdU according to BrdU flow kit protocol.

Adoptive Transfer and Recall of Memory CD8$^+$ T Cells. WT (CD45.1) and CD25-deficient (CD45.2) memory CD8$^+$ T cells from chimeric mice that were infected more than 30 days previously with 10^3 PFU of Lm-ova were purified by magnetic bead enrichment followed by cell sorting on a FACSAria to greater than 98% purity. One thousand Ova/K^b-specific memory CD8$^+$ T cells from each cell population were mixed together and injected i.v. into naïve CD45.1/CD45.2 recipient mice. One day later mice were challenged with 5×10^3 PFU of LM-ova and the number of tetramer$^+$ cells was enumerated in the spleen and lungs on day 8 post-challenge.

Statistical Analysis. Statistical significance was determined by a Student's *t* test using Prism 5. Significance was set as any *P* value less than 0.05.

1. Blattman JN, et al. (2003) Therapeutic use of IL-2 to enhance antiviral T-cell responses in vivo. *Nat Med* 9:540–547.
2. Masopust D, Vezys V, Marzo AL, Lefrançois L (2001) Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 291:2413–2417.
3. Altman JD, et al. (1996) Phenotypic analysis of antigen-specific T lymphocytes. *Science* 274:94–96.
4. Obar JJ, Khanna KM, Lefrançois L (2008) Endogenous naive CD8+ T cell precursor frequency regulates primary and memory responses to infection. *Immunity* 28: 859–869.
5. Fuse S, et al. (2009) Recall responses by helpless memory CD8+ T cells are restricted by the up-regulation of PD-1. *J Immunol* 182:4244–4254.

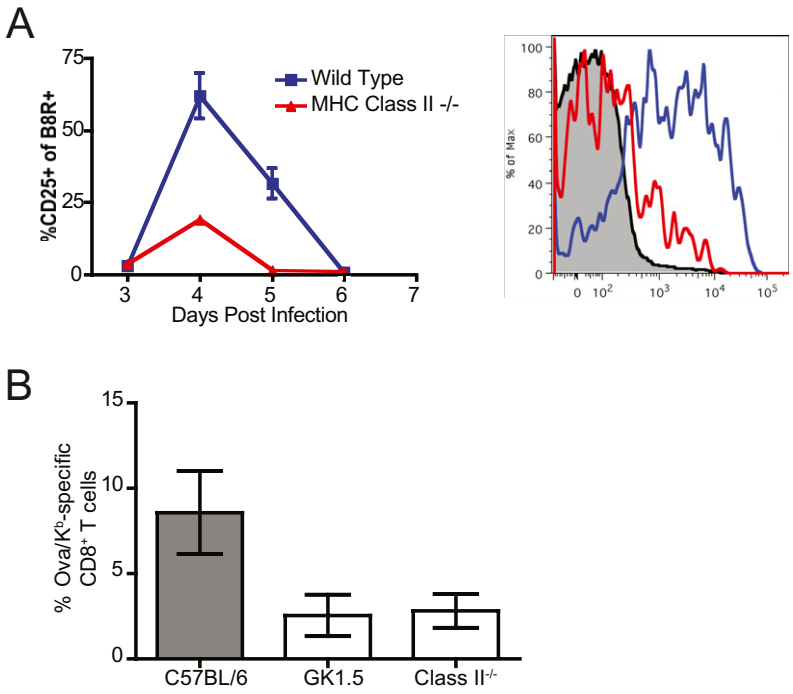

Fig. S1. Class II-deficient mice exhibit a similar phenotype to anti-CD4 treated C57BL/6 mice. (*A*) C57BL/6 or Class II-deficient mice were infected with 2×10^6 PFU of VV-WR and CD25 expression on VV-specific splenic $CD8^+$ T cells was monitored longitudinally at the indicated times (*Left*) or three days after VV infection (*Right*). Data from C57BL/6 mice is shown blue, whereas Class II-deficient mice are displayed in red. (*B*) C57BL/6, anti-CD4 treated, or Class II-deficient mice were infected with 10^3 CFU of LM-ova and the expansion of Ova/K^b-specific $CD8^+$ T cells in the spleen was determined nine days later. These data are representative of two independent experiments, each containing four to five mice per group.

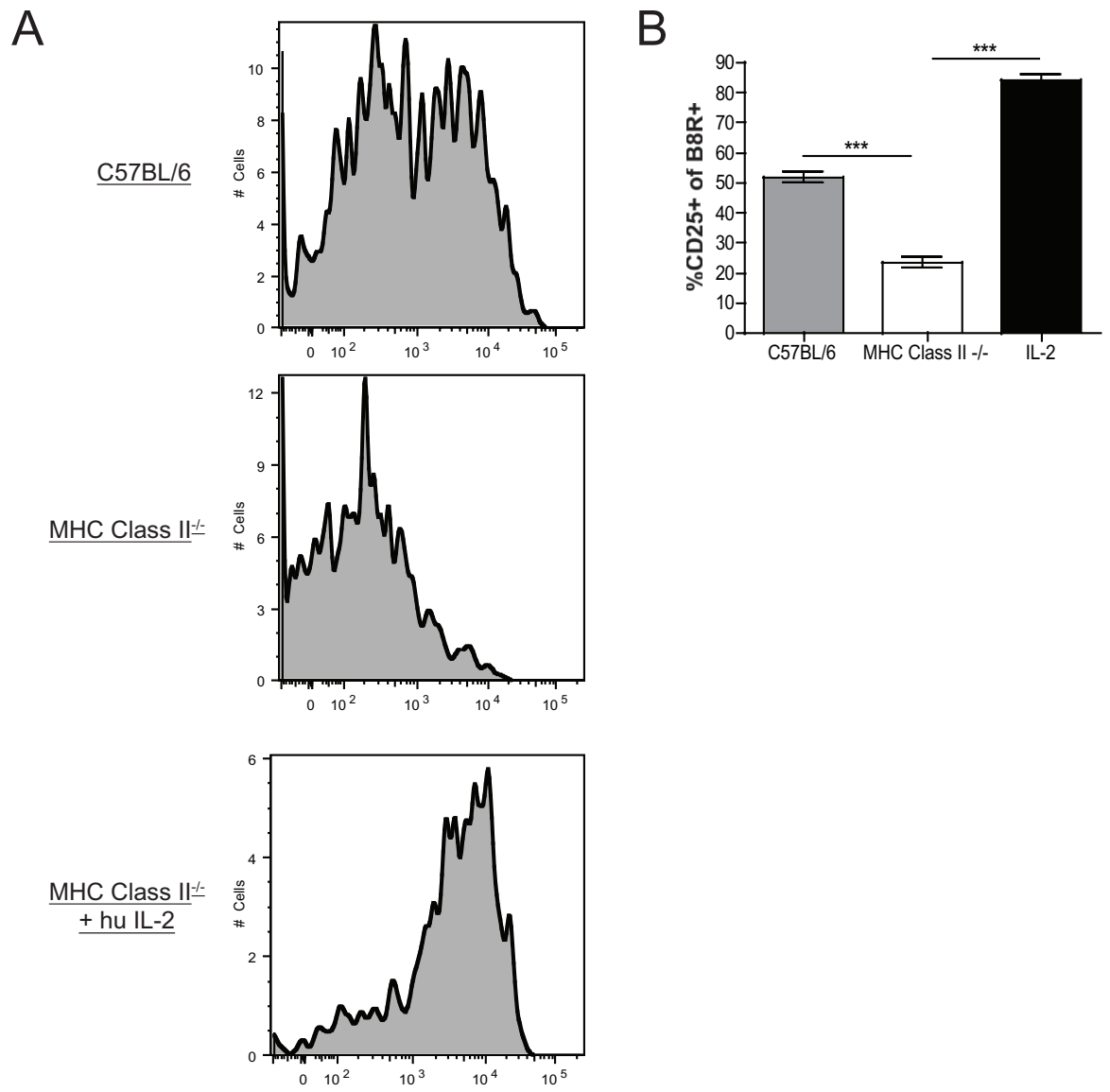

Fig. S2. rIL-2 rescues CD25 expression on VV-specific CD8$^+$ T cells. C57BL/6 or Class II-deficient mice were infected with 2×10^6 PFU of VV-WR. Additionally, a group of Class II-deficient mice were treated with 15,000U of rIL-2 twice a day from day 1 to 4. At which point, expression of CD25 on the VV-specific CD8$^+$ T cells in the spleen was monitored. Representative histograms (*A*) or mean percent CD25+ $\pm$ 1 SD from four mice per group were graphed (*B*). Statistical significance was determined using a Student's *t* test.

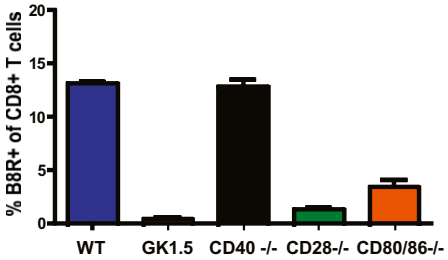

Fig. S3. VV-specific CD8$^+$ T cell expansion is impaired in helpless, CD28-deficient, and CD80/86-deficient mice, but not CD40-deficient mice. Mice were infected with 2×10^6 PFU of VV-WR and subsequent expansion of the VV-specific CD8$^+$ T cells was determined seven days after infection in the spleen. The graph displays the mean percent B8R/K^b-specific CD8$^+$ T cells $\pm$ 1 SD with C57BL/6 (blue), CD4-depleted WT mice (red), CD40$^{-/-}$ mice (black), CD28$^{-/-}$ mice (green), or CD80/86$^{-/-}$ mice (orange).

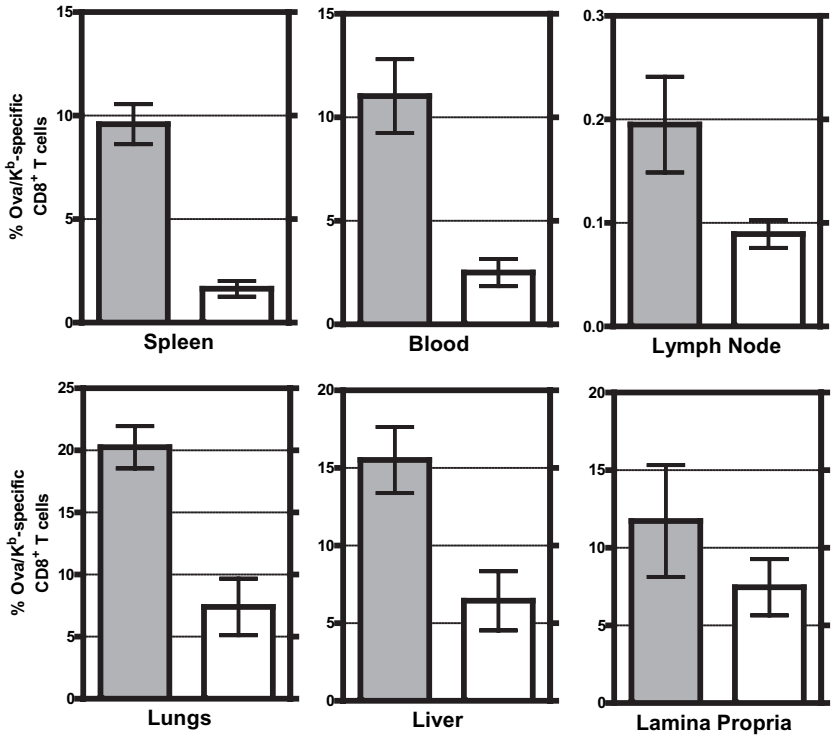

Fig. S4. CD25-mediated signals are necessary for the accumulation of pathogen-specific CD8$^+$ T cells in lymphoid and nonlymphoid tissues. CD25$^{-/-}$ /WTmixed chimera mice were infected with 10^3 CFU of Lm-ova and nine days later the magnitude of the Ova/K^b-specific CD8$^+$ T cell response was evaluated. Graphs display the percentage of Ova/K^b-specific CD8$^+$ T cells in either the WT (filled bar) or CD25$^{-/-}$ (open bar) compartment. These data are representative of two independent experiments containing three to five mice.

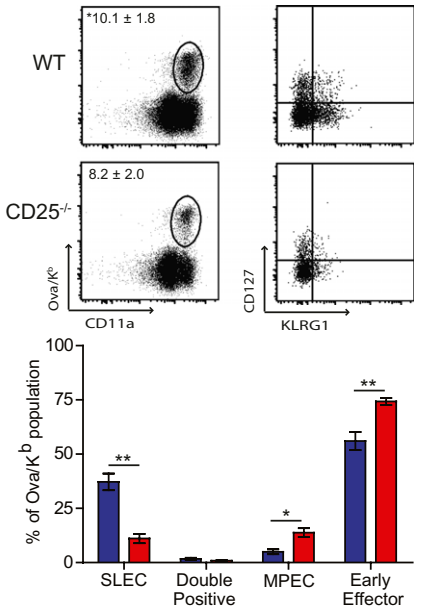

Fig. S5. CD25 regulates SLEC development in the CD8 response to VSV infection. CD25$^{-/-}$/WT mixed chimera mice were infected with 10^5 PFU of VSV-ova and seven days later the magnitude of the Ova/K^b-specific CD8$^+$ T cell response was evaluated. Values indicate the percentage of tetramer$^+$ cells among CD8$^+$ T cells. Graphs display the percentage of Ova/K^b-specific CD8$^+$ T cells in either the WT (blue bar) or CD25$^{-/-}$ (red bar) compartment. These data are representative of two independent experiments containing three to five mice.

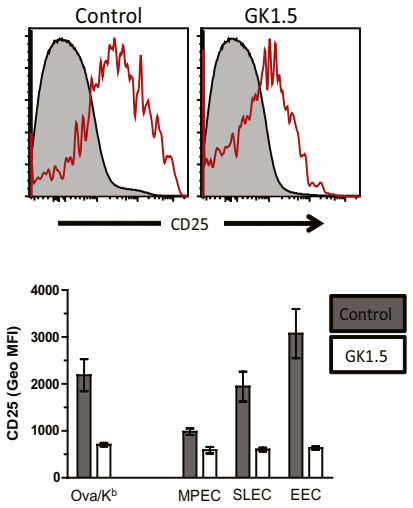

Fig. S6. CD25 expression levels of effector CD8$^+$ T cell populations. C57BL/6 mice were either treated with anti-CD4 or left untreated. Mice were subsequently infected with 10^3 CFU of Lm-ova and expression of CD25 on pathogen-specific CD8$^+$ T cells was determined four days after infection in the spleen. The histograms (*Top*) display representative CD25 expression from either untreated or CD4-depleted mice. The filled histogram displays CD25 expression from bulk naïve CD8$^+$ T cells (CD11a^{low} CD44^{low}) and the open histogram is gated on Ova/K^b-specific CD8$^+$ T cells. The graph (*Bottom*) displays the geometric MFI of CD25 on either the bulk Ova/K^b-specific CD8$^+$ T cell population or on each of the effector CD8$^+$ T cell populations (EEC = KLRG1^{low} CD127^{low}; SLEC = KLRG1^{high} CD127^{low}; MPEC = KLRG1^{low} CD127^{high}).

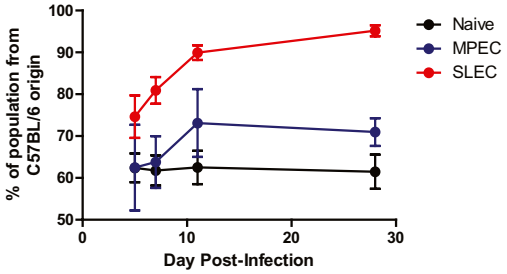

Fig. S7. CD25-deficient SLEC CD8$^+$ T cells are under-representated late during infection. WT:CD25$^{-/-}$ mixed chimera mice were infected with 10^3 CFU of LM-ova. At the indicated times, Ova/K^b-specific CD8$^+$ T cell were indentified and then the percentage of that population which is of WT and CD25$^{-/-}$ origin was enumerated by congenic markers. The graph displays the percentage of the populations that is of WT origin. The black depicts the bulk naïve CD8+ T cell population (CD11a^{low} tetramer^{neg}), whereas the Ova/K^b-specific SLEC and MPEC populations are shown in red and blue, respectively. A value higher than the naïve CD8$^+$ T cell value indicates the WT (C57BL/6) is over-representative in the population.