SUPPLEMENTAL MATERIAL

Soroosh et al., http://www.jem.org/cgi/content/full/jem.20101562/DC1

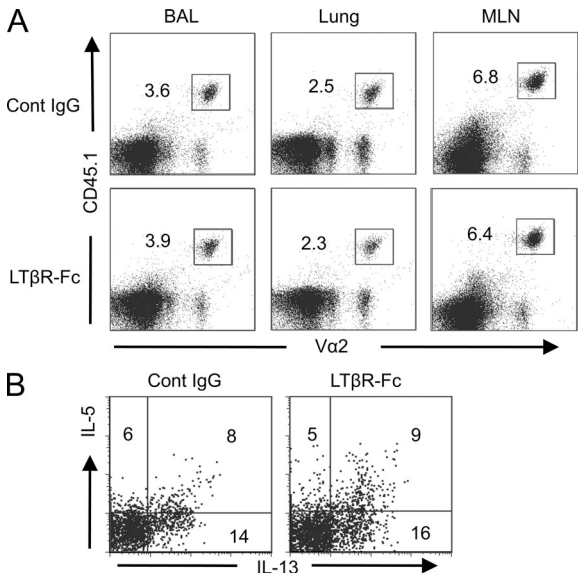

Figure S1. LT-βR–Fc treatment does not affect accumulation or cytokine secretion of Th2 cells immediately after recall antigen. (A) In vitro generated OVA-specific CD45.1 OT-II Th2 cells were transferred into CD45.2+ WT mice, which were subsequently exposed to soluble OVA i.n. and treated with control (Cont) IgG or LT-βR–Fc on a consecutive 3 d. The percentage of donor Th2 cells (CD45.1+Vα2+) was analyzed on day 4, 24 h after the last OVA challenge in BAL, lungs, and MLNs. (B) Recipient mice were left for several weeks before another rechallenge with OVA, as described in Fig. 1. MLN cells were restimulated in vitro with OVA peptide for 8 h. IL-5 and IL-13 production by long-lived CD45.2+ donor cells was analyzed by ICS. Results are representative of two independent experiments.

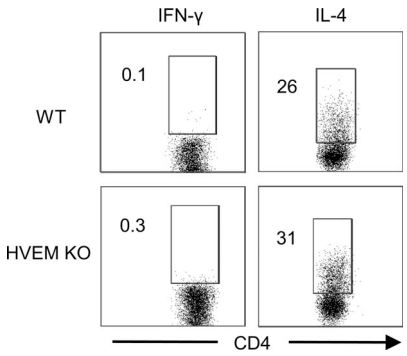

Figure S2. Cytokine profile of Th2 cells before transfer. In vitro generated WT and HVEM$^{-/-}$ OT-II Th2 cells were restimulated with OVA peptide–pulsed T cell–depleted APCs for 8 h. IFN-γ and IL-4 production by CD4 T cells was analyzed by ICS. Results are representative of three independent experiments.

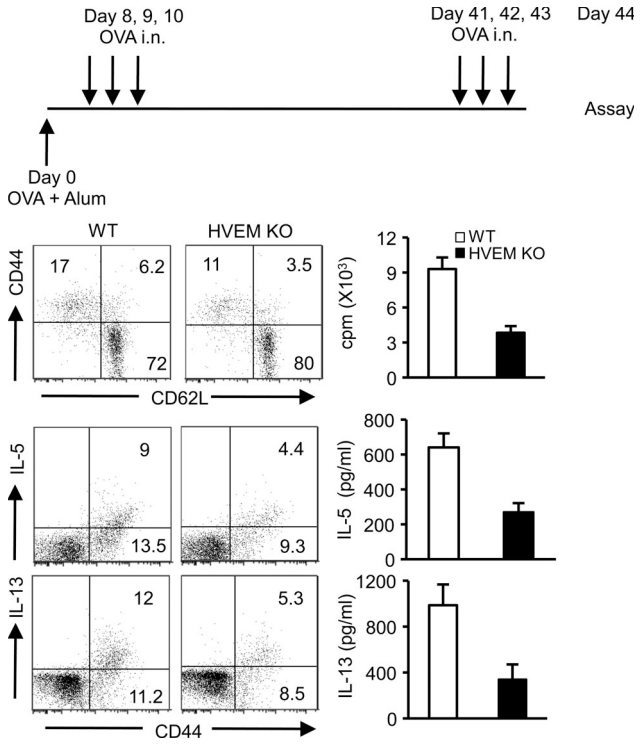

Figure S3. HVEM signals support optimal generation of endogenous polyclonal memory Th2 populations. WT and HVEM$^{-/-}$ mice were immunized with 50 µg OVA plus alum on day 0 and then challenged with soluble OVA i.n. on a consecutive 3 d. 4 wk later, all mice were rechallenged i.n. with OVA on a consecutive 3 d. MLNs were collected 24 h after the last OVA challenge. Expression of CD44 and CD62L was analyzed on total gated CD4 T cells (top left). Single cell suspensions from the LNs were cultured with 100 µg/ml OVA protein for 2 d. OVA-specific IL-5 and IL-13 production by CD44-stained CD4 T cells was analyzed by ICS (middle and bottom left). OVA-specific proliferation (top right) and Th2 cytokine production in supernatants (middle and bottom right) were also analyzed. Results are from four individual mice per group (either representative flow plots [left] or means ± SD [right]). Data are representative of two independent experiments.

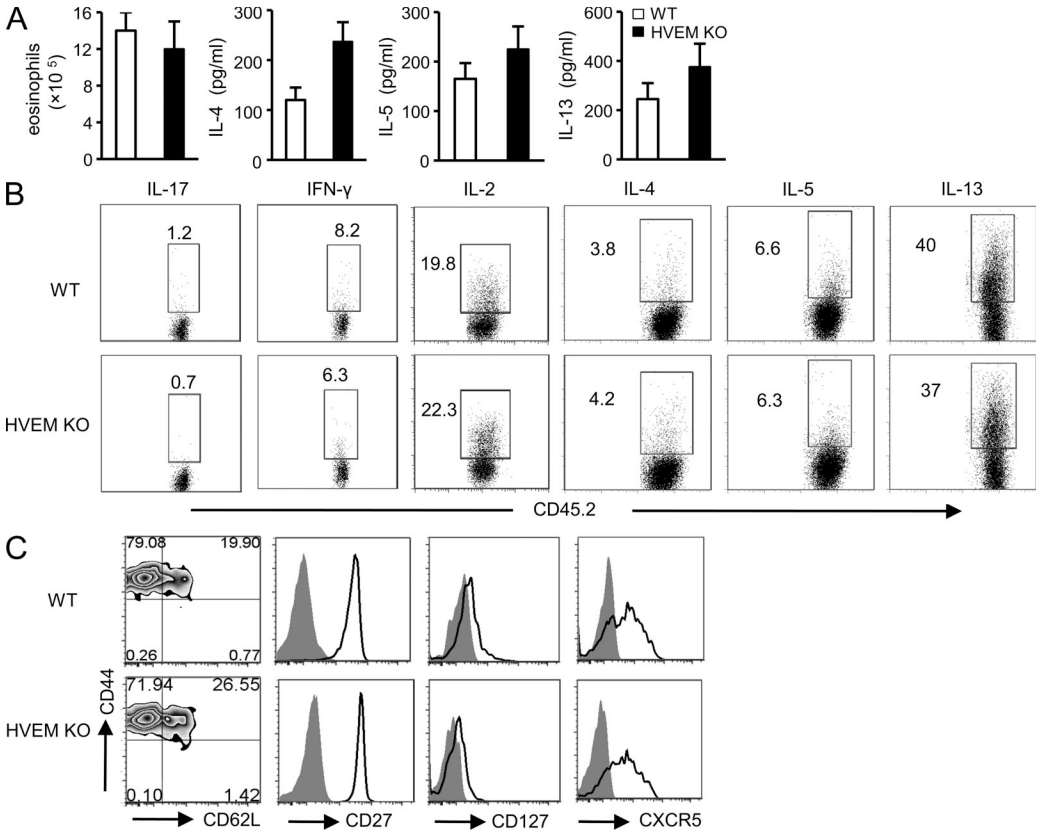

Figure S4. HVEM is not required for the differentiation and effector function of Th2 cells. Th2 cells were generated in vitro from CD45.2+ WT or HVEM$^{-/-}$ OT-II mice as in Fig. 2. Rested Th2 cells were transferred i.v. into congenic CD45.1+ WT mice, which were challenged with soluble OVA i.n. on a consecutive 3 d. Samples were collected on day 4, 24 h after the last OVA challenge. (A) Numbers of eosinophils and levels of Th2 cytokines (IL-4, IL-5, and IL-13) in BAL of recipient mice on day 4. Results are the mean ± SD from six to eight mice per group. (B) MLN cells from the recipient mice were re-stimulated in vitro with OVA peptide for 8 h. IFN-γ, IL-2, IL-4, IL-5, IL-13, and IL-17 production by gated CD45.2+ donor cells was analyzed by ICS. (C) Surface phenotype of the responding gated CD45.2+ WT and HVEM$^{-/-}$ Th2 cells, without in vitro restimulation, analyzed 4 d after initial in vivo antigen challenge. Closed histograms show isotype control staining. Results are representative of two independent experiments.

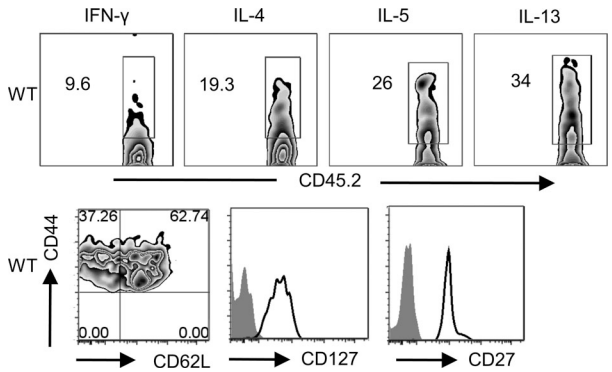

Figure S5. Cytokine profile and the phenotype of memory Th2 cells. WT and HVEM$^{-/-}$ Th2 cells were transferred to congenic hosts and challenged with OVA i.n. 40 d later, MLNs were collected. IFN-γ, IL-4, IL-5, and IL-13 production by gated WT CD45.2+ donor cells was analyzed by ICS after in vitro restimulation with OVA for 8 h (top). Surface phenotype of the recovered WT donor T cells was assessed without stimulation (bottom). Sufficient numbers of HVEM$^{-/-}$ T cells were not recovered for analysis. Closed histograms show isotype control staining. Results are representative of two independent experiments.

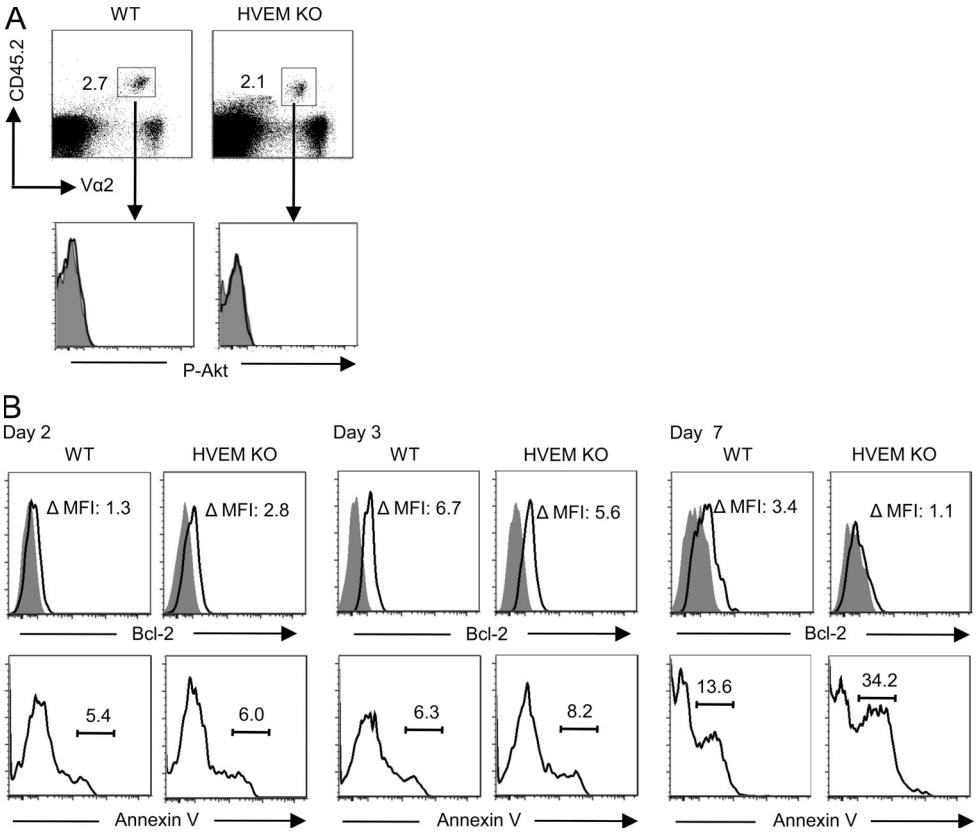

Figure S6. Reduced Bcl-2 expression and increased apoptosis of Th2 cells in the absence of HVEM on T cells. In vitro generated WT and HVEM$^{-/-}$ OT-II Th2 cells were transferred into congenic CD45.1^{+} WT mice, which were subsequently challenged with OVA i.n. on a consecutive 3 d as in Fig. 4 C. MLNs were collected on days 2, 3, and 7. (A) At day 7, donor Th2 cells were stained for intracellular phosphorylated Ser-473 Akt (pAkt). (B) At days 2, 3, and 7, cells were stained for surface CD45.2, Vα2, and intracellular Bcl-2 or annexin V (open histogram). Closed histograms show isotype control staining. MFI, mean fluorescent intensity.

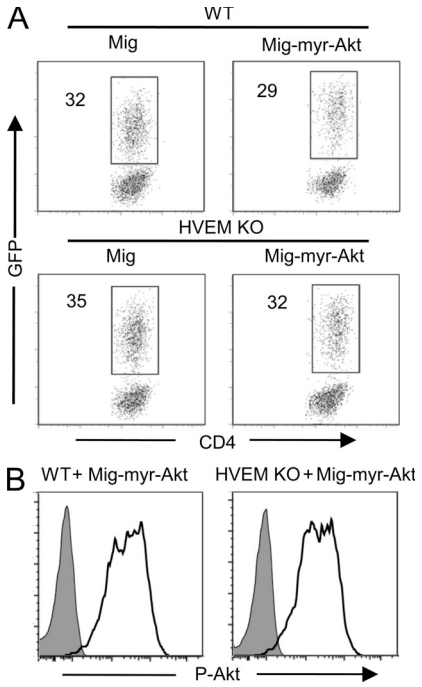

Figure S7. Retroviral transduction of Th2 cells with active Akt. Naive CD4+ T cells from WT or HVEM$^{-/-}$ OT-II mice were stimulated under Th2 conditions and transduced with retroviral vectors expressing either GFP alone (Mig) or GFP with myristoylated Akt (Mig-myr-Akt). (A) Expression of GFP in transduced CD4 T cells on day 7 before transfer into recipient mice. (B) Phosphorylated Ser-473 Akt (pAkt) on GFP+ CD4 T cells detected by ICS on day 7. Closed histograms show isotype control staining. Results are representative of two independent experiments.

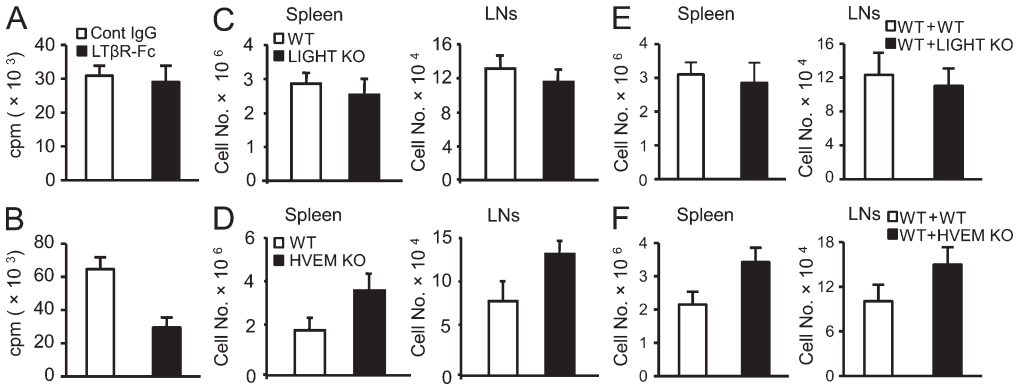

Figure S8. LIGHT and HVEM do not affect primary expansion of CD4 T cells. (A) Purified naive CD4+ cells from WT OT-II mice were stimulated with plate-bound anti-CD3 in the absence of APCs. LT-βR–Fc or human IgG (cont IgG) was added as indicated. 3 d later, [3H]thymidine incorporation was measured as an indicator of proliferation. (B) Naive CD4+ T cells were stimulated for 4 d as in A in the presence of LT-βR–Fc or control Ig. Activated T cells were washed with fresh medium, and equal numbers of T cells were restimulated with 1 µg/ml plate-bound anti-CD3 for an additional 2 d. [3H]thymidine incorporation was measured. Results are representative of least three independent experiments. (A and B) Mean (±SD) of triplicate cultures is shown. (C–F) Purified naive CD45.2+ WT, LIGHT$^{-/-}$, or HVEM$^{-/-}$ OT-II cells were adoptively transferred alone (C and D) or with an equal number of naive CD45.1+ WT OT-II cells (E and F) into congenic WT CD45.1+ mice, as in Fig. 7. Recipients were immunized with OVA, and 3 d later, the absolute number of donor CD45.2+ T cells was determined in the spleen and LNs of recipient mice. Results are the mean ± SD from four individual mice per group and representative of two independent experiments.