Supporting Information

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SI Text

Generation of Mice with CD4-Specific Deletion of Th2 LCR. RHS4,5,7 knockout (KO) mice were generated as described elsewhere (manuscript in preparation) by targeting the RHS4,5KO construct into RHS7KO ES cells (1) (Fig. S1*A*). By crossing the RHS4,5,7KO-Neo mice with Cre transgenic mice, we generated RHS4,5,7$^{+/-}$ or LCR$^{+/-}$ mice (Fig. S1*A*). Crossing LCR heterozygote mice produced no viable homozygous knockout mice, suggesting that these mice are embryonic lethal, presumably because the deletion of the entire LCR also causes deletion of *rad50* gene exons 22–24 (Fig. S1*A*) and *rad50* is an essential gene (2).

To generate mice with CD4-specific deletion of LCR, we crossed the RHS4,5,7KO mice with CD4-Cre transgenic mice on an LCR$^{+/-}$ background. In this type of cross, some progeny will have one locus that has deleted the LCR and the other locus has deleted RHS4, RHS5, and RHS7 (Fig. S1*B*). In mice of this genotype that have the CD4-Cre transgene(s), the region between RHS4 and RHS7 on the chromosome from the RHS4,5,7KO will be deleted by Cre-LoxP-mediated recombination in a T cell-specific manner, because of the residual LoxP sites that were introduced for the construction of the RHS4,5,7 chromosome (Fig. S1*B*). PCR genotyping of the CD4 T cells shows that the vast majority of these cells had, in fact, deleted the LCR (Fig. S1*C*). The resulting LCR$^{-/-}$ CD4 T cells were viable and were present in a similar number as wild-type CD4 T cells (Fig. S3).

Evaluation of Functional Defects in the Differentiation of CD4 T Cells from cLCR KO Mice. The number of naïve CD4 T cells from cLCR KO mice was comparable to that from wild-type mice (Fig. S4*A*). In addition, the number of in vitro-stimulated Th1 cells was comparable between wild-type and cLCR KO mice (Fig. S4*B*). However, the number of in vitro-stimulated (primary stimulation) Th2 cells from cLCR KO mice was decreased by ~40% compared to those from wild-type mice (Fig. S4*B*). The number of restimulated Th2 cells from cLCR KO mice was decreased by ~50% compared to that from wild-type mice (Fig. S4*B*). However, this lower cell number does not explain the reduction of Th2 cytokines in cLCR KO mice (Fig. 1), because at the time of restimulation, we plated the same number of primary-stimulated cells to measure cytokine production in ELISA and RT-PCR (Fig. 1). The numbers of effector/memory cells in vivo were also comparable between wild-type and cLCR KO mice (Fig. S4*C*). In addition, we compared the numbers of effector/memory cells in OVA-sensitized and challenged mice. The number of effector/memory cells in these mice was also comparable between wild-type and cLCR KO mice (Fig. S4*D*). We next examined whether the effector/memory cells present in cLCR KO mice were preferentially selected because their LCRs were not deleted because of incomplete Cre-loxP recombination. If this were the case, these cells would have selective advantage in survival during growth and differentiation. We examined this possibility by measuring deletion of the Th2 LCR in these cells by PCR. The majority of in vitro-stimulated and restimulated cells and in vivo effector/memory cells from cLCR KO mice retained the deletion in the Th2 LCR (Fig. S5), which excluded this possibility.

Procedures for Animal Model of Allergic Asthma. ***Sensitization and challenge.*** Five- to 7-week-old C57BL/6 wild-type and cLCR KO mice were sensitized by i.p. injection of 20 μg of OVA (Sigma) with 100 μL of aluminum hydroxide gel in PBS on day 0 and day 5. Control mice were challenged with aluminum hydroxide gel and PBS. One week after the second immunization, mice received an aerosol challenge for 5 consecutive days (days 12–16) for 40 min each day with 1% OVA (wt/vol) in endotoxin-free PBS or endotoxin-free PBS alone. Twenty-four hours after the last exposure, the mice were killed for analysis. Experiments with live mice were approved by the Sogang University Institutional Animal Care and Use Committee.

Total and differential cell counting of BAL fluid. BAL fluid was obtained by washing the lung twice with 0.8 mL of PBS and was kept on ice. For total cell counting, the cells in the BAL fluids were pelleted by centrifugation at 3,600 rpm for 5 min at 4 °C and resuspended in 0.1 mL of PBS. The total number of cells was counted with a hemocytometer. For differential cell counting, BAL fluids cells were pelleted onto a glass slide at 1,500 rpm for 5 min at room temperature using cytospin (Hettich). Cells on the slides were stained with Diff-Quik solution (Sysmex), and cell types were determined by observing cell morphology and staining patterns under a microscope.

Measurement of serum IgE. Blood was obtained from the hearts of anesthetized mice and stored in a microtube overnight at room temperature. Serum was obtained from the blood by centrifugation at 14,000 rpm for 15 min. The level of IgE was measure by ELISA.

Lung histology. The lungs from OVA-challenged wild-type or cLCR KO mice were fixed in 10% neutral buffered formalin overnight and dehydrated with ethanol before paraffin embedding. Paraffin-embedded tissues were sectioned using microtome. Thin sections were hydrated by sequential treatment of xylene, 100%, 85%, and 70% ethanol and water, and stained with hematoxylin and eosin for airway inflammation, or with periodic acid-schiff (PAS) for mucus production.

Airway hyperresponsiveness. Airway hyperresponsiveness was measured as a change in airway resistance (cm H_2O/mL/s) after challenge with aerosolized methacholine. Anesthetized (pentobarbital sodium, 70–90 mg/kg, intraperitoneal), tracheostomized (stainless-steel cannula, 18G) mice were mechanically ventilated, and lung function was assessed. Lung resistance (R_L) and compliance were measured using the FlexiVent system (SCIREQ).

1. Lee GR, Spilianakis CG, Flavell RA (2005) Hypersensitive site 7 of the TH2 locus control region is essential for expressing TH2 cytokine genes and for long-range intrachromosomal interactions. *Nat Immunol* 6:42–48.

2. Luo G, et al. (1999) Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc Natl Acad Sci USA* 96:7376–7381.

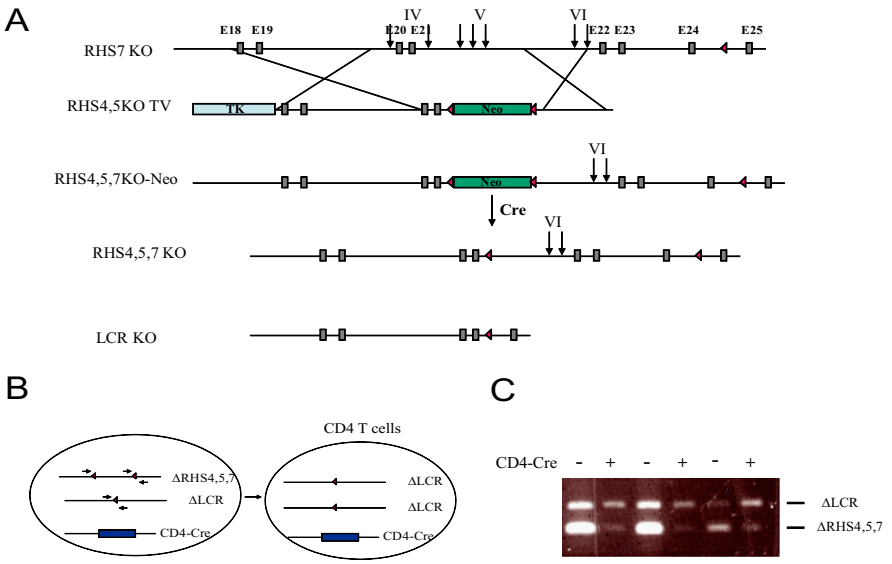

Fig. S1. Strategy for making CD4-specific LCR KO mice. (*A*) RHS4,5KO targeting vector was transfected into RHS7KO ES cells to get RHS4,5,7KO-Neo mice. RHS4,5,7$^{+/-}$ or LCR$^{+/-}$ mice were generated by crossing the RHS4,5,7KO-Neo mice with Tet-Cre transgenic mice. (*B*) RHS4,5,7$^{-/-}$ mice were crossed with LCR$^{+/-}$-CD4-Cre^{+} transgenic mice to generate CD4 conditional LCR KO mice. (*C*) The primers for PCR are shown in *B*. The upper band represents ΔLCR, and the lower band represents ΔRHS4,5,7.

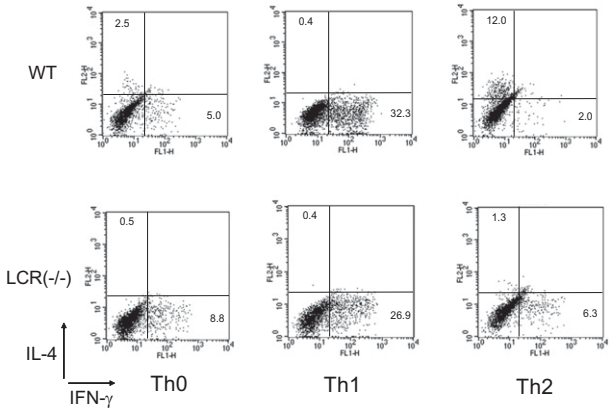

Fig. S2. Intracellular staining of IL-4 and IFN-γ in Th0, Th1, and Th2 cells from wild-type and CD4 conditional LCR$^{-/-}$ mice. Naïve CD4 T cells were stimulated with Th0, Th1, or Th2 conditions for 5 days. Cells were restimulated with ionomycin plus PMA for 5 h and then stained for intracellular IL-4 and IFN-γ.

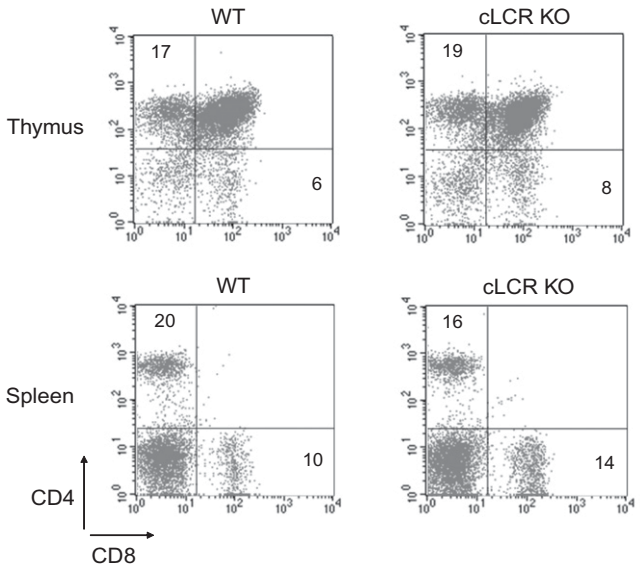

Fig. S3. Ratio of CD4 and CD8 cells from wild-type or cLCR KO mice. Cells in the thymus and spleen were isolated, stained with anti-CD4 and anti-CD8 antibodies, and analyzed by FACS.

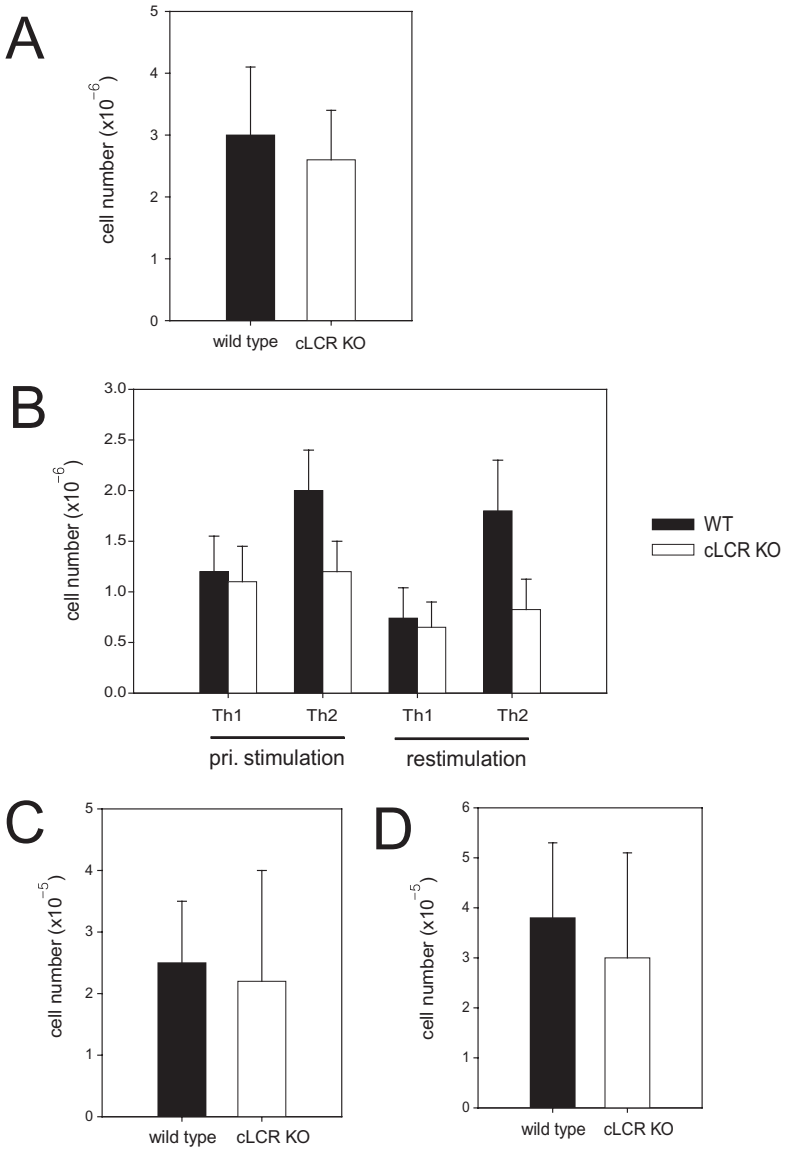

Fig. S4. Numbers of naïve and effector/memory CD4 T cells from wild-type and cLCR KO mice. (*A*) Numbers of naïve CD4 T cells from wild-type and cLCR KO mice. Naïve cells were isolated from the spleen and stained with CD62L^{high} and CD44^{low} ($n = 4$). (*B*) Naïve CD4 T cells (1×10^6 cells) were primarily stimulated for 4 days and restimulated in vitro under Th1 or Th2 polarizing conditions. Cell numbers were counted after 4 days (primary stimulation). Primarily stimulated cells were then restimulated for 12 h. Experiments were done in triplicate. (*C*) Numbers of in vivo effector/memory CD4 T cells from wild-type and cLCR KO mice. Effector/memory cells were isolated from spleens and counted based on CD62L^{low} and CD44^{high} staining ($n = 4$). (*D*) Numbers of in vivo effector/memory CD4 T cells from OVA-sensitized and challenged wild-type and cLCR KO mice. Effector/memory cells were isolated from spleens and counted based on CD62L^{low} and CD44^{high} staining ($n = 4$).

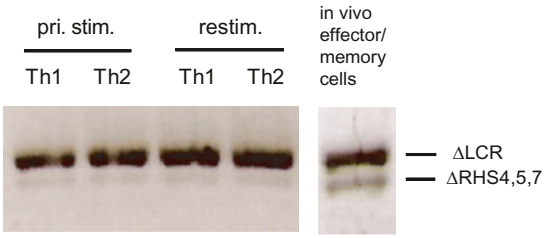

Fig. S5. Deletion of Th2 LCR in in vitro-stimulated and in vivo effector/memory cells. Naïve CD4 T cells were isolated from cLCR KO mice and primarily stimulated and restimulated under Th1 or Th2 polarizing conditions or in vivo effector/memory cells were isolated from cLCR KO mice as in Fig. S2. DNAs were isolated from these cells, and they were analyzed by PCR using primers described in Fig. 1 *B* and *C*. The upper band represents ΔLCR, and the lower band represents ΔRHS4,5,7.

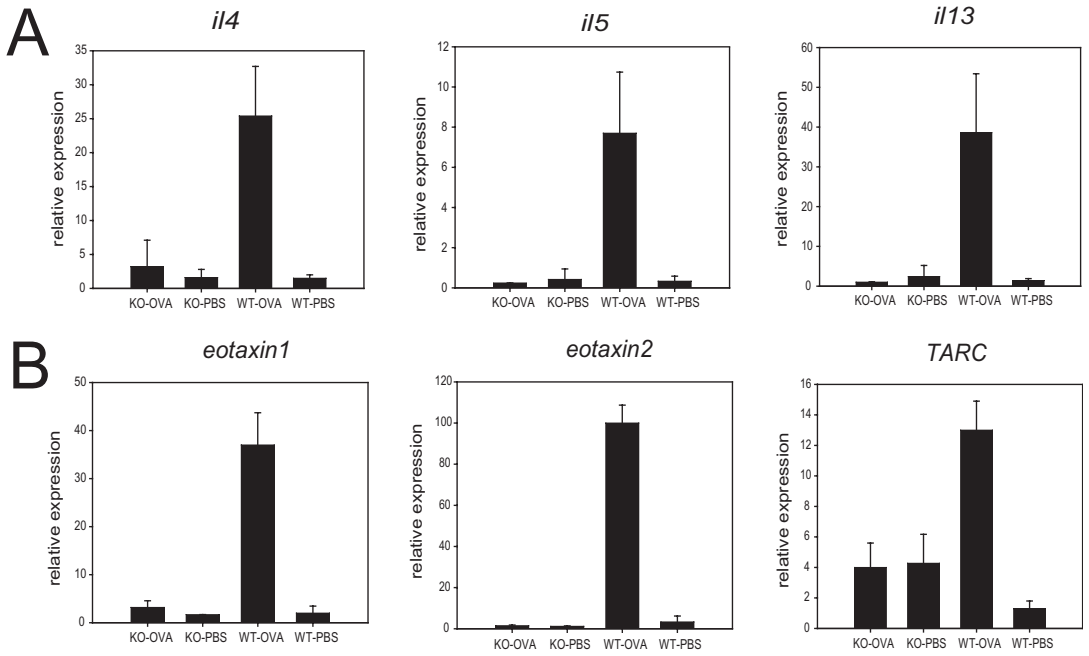

Fig. S6. Expression of Th2 cytokines and chemokines in the lung. Wild-type or cLCR KO mice were sensitized and challenged with OVA or PBS as described in *Materials and Methods*. Total RNA was isolated from the lung and subjected to quantitative RT-PCR.

Table S1. Primers for quantitative PCR

Primer name	Sequence
IL-4 promoter Fw	GCAGGATGACAACTAGCTGGG
IL-4 promoter Rv	ACGGCACAGAGCTATTGATGG
IL-5 promoter Fw	TTTCCTCAGAGAGAGAATAAATTGCTT
IL-5 promoter Rv	GCTGGCCTTCAGCAAAGG
IL-13 promoter Fw	ACCCAGAACCTGGAAACCCT
IL-13 promoter Rv	GTGGCCGCTAAAGGAAAGAGT
CNS-1 Fw	CACACACTGGTCCACTGTGATG
CNS-1 Rv	GACGCAGGCACCAAAATTAAA
CNS-2 Fw	ATCACGTCGTCTTACCCAAACA
CNS-2 Rv	TGTGGGAGAGCGTCTGATCTG
HSVa Fw	CCCTCCATACAGTTCCTGCAGTA
HSVa Rv	ACCAGGGCACTTAAACATTGCT
β-Globin Fw	AGATTTTTCCACTCCCTATTC
β-Globin Rv	TGGCTGTAAGAAACCTAAATTAG