Supporting Information

Hwang et al. 10.1073/pnas.1214682110

SI Materials and Methods

Mice. Six- to eight-week-old C57BL/6 mice were purchased from Orient Bio and maintained in the Sogang University animal facility under specific-pathogen free conditions. The generation of RHS7-deficient mice (1), pSico-YY1 transgenic mice (2), and GATA3$^{flox/-}$ mice (3) has been described previously. CD4-Cre transgenic mice were purchased from Taconic. Experiments with live mice were approved by the Sogang University Institutional Animal Care and Use Committee.

EMSA. Preparation of nuclear extracts has been described previously (4). The oligonucleotides sequences used are as follows. RHS7b: 5′-ATGAGCAAAAATGTCCCCATACACCCACTC-3′; RHS7b-mutant: 5′-ATGAGCAAAAATGT*AAA*CATACACCCACTC-3′; YY1-consensus: 5′-TGCCTTGCAAAATGGCGTTACTGCAG-3′. The core YY1-binding sequence is underlined.

ChIP–Quantitative PCR. Cells (1×10^7) were cross-linked with 1% paraformaldehyde on ice for 30 min and quenched with 0.125 M glycine. Cells were then lysed with a buffer containing 1% SDS and sonicated at the high-power setting for 15 min using a Bioruptor sonicator (Diagenode). Cell extracts were precleared with protein A/G agarose/salmon sperm DNA (Upstate) and incubated with a mixture of anti-trimethyl-H3K4 and anti-monomethyl H3K4 (Millipore 07-473 and 07-436, respectively), anti-acetyl H3 (Upstate 06-599), anti-GATA3 (Santa Cruz, sc-268ac), anti-YY1 (mixture of Santa Cruz sc-1703×, sc-7341×, and Abcam 12132), or normal rabbit IgG or normal mouse IgG (Santa Cruz) as a negative control. Antibody-bound chromatin was precipitated by protein A/G agarose, washed, and eluted with a buffer containing sodium bicarbonate-SDS. The chromatin was reverse cross-linked by incubating at 65 °C for 4 h, followed by protease K treatment. The amount of precipitated DNA was quantified by quantitative PCR using the SYBR green (Kappa Bio) method with the primers listed in Table S1.

In Vitro Differentiation of CD4 T Cells. Isolation and in vitro differentiation of CD4 T cells into Th1 and Th2 cells has been described previously (1).

RNA Isolation and Quantitative RT-PCR for Cytokines or Chemokines. Total RNA isolation and quantitative RT-PCR has been described previously (1). Relative amounts of expression were normalized by the amount of *hprt* or *actb*. The sequences used for quantitative PCR are listed in Table S2.

Immunoblot Analysis. Cell extracts were resolved on 10% (wt/vol) SDS/PAGE and transferred to a PVDF membrane (Bio-Rad). The membrane was blocked with 5% (wt/vol) skim milk in Tris-buffered saline with Tween (TBST, 50 mM Tris, 150 mM NaCl, 0.05% Tween20; pH 7.6) (incubated 1 h at room temperature). The membrane was then probed with the antibody against YY1 (Santa Cruz, sc-1703), GATA3 (Santa Cruz, sc-268), or β-Actin (Santa Cruz, sc-47778), diluted 1:100 or 1:1,000 in TBST overnight at 4 °C. Then, an HRP-conjugated antibody against rabbit or mouse or goat IgG (Biolegend) diluted at 1:2,000 in 5% skim milk TBST was added for 1 h at room temperature. The signal was detected on an X-ray film by ECL reaction.

Retroviral Transduction. Mouse *yy1* cDNA was cloned into MIEG3 retroviral vector. A total of 1–2 × 10^6 Phoenix Eco cells were transfected with empty MIEG3 or MIEG3-*yy1* in combination with the pCL-Eco helper vector. A culture supernatant containing high titers of retrovirus was collected after 48 h of transfection. Purified naive CD4 T cells were activated with plate-bound anti-CD3 (5 μg/mL) and mIL-2 (1 μg/mL) for 24 h. Next, activated T cells were spin-infected in 1 mL of retrovirus-containing supernatant with polybrene (4 μg/mL) at 1,500 × *g* for 90 min at 32 °C. After the spin, infected cells were incubated for 3 d in a Th1 or Th2 polarizing condition. Then, cells were restimulated for intracellular cytokines analysis, or GFP$^+$ cells were sorted and restimulated with anti-CD3 for 4 h for quantitative RT-PCR.

Intracellular Cytokine Staining. Cells were restimulated with 1 μM ionomycin (Sigma) and 10 nM PMA (Sigma) with Golgi stop (BD) for 4 h. Intracellular staining was performed using Cytofix/cytoperm kit (BD) according to the manufacturer's directions. Flow cytometric analysis was done using FACS Caliber (BD).

Cell Transfection and Dual Luciferase Assay. Expression vectors for *gata3* and *yy1* were constructed from the CMV-base expression vector (pCMVSPORT6). Cell transfection into EL4, a mouse thymoma cell line, and measurement of dual luciferase was performed as previously described (4) with minor modifications. Transfection efficiency was normalized by dividing *Firefly* luciferase activity by *Renilla* luciferase activity.

DNA Methylation Analysis. DNA methylation analysis using bisulfate modification was performed as described (1).

Chromosome Conformation Capture Assay. Chromosome conformation capture (3C) analysis was performed as described previously (5) with minor modification. The purified DNA was amplified using the TaqMan qPCR method. Primers used are listed in Table S3.

Coimmunoprecipitation. HEK 293T cell were transfected with pCMV-*yy1* and pCMV-*gata3*. Two days after transfection, cell lysates were made with a lysis buffer and then precleared with control IgG followed by protein A/G (Santa Cruz) treatment. Precleared lysates were incubated overnight at 4 °C with monoclonal anti-GATA3, polyclonal anti-YY1, or normal IgG. Then, protein A/G beads were added, followed by incubation for an additional 2 h. Immunocomplexes were washed and resuspended in an SDS loading buffer. Immunoblot analysis was performed as described previously.

Immunofluorescence Assay and in situ Proximity Ligation Assay. CD4 T cells were fixed onto poly-L-lysine-coated coverglass (BD Science) in 2% paraformaldehyde for 15 min at room temperature, washed with PBS, and permeabilized with 0.5% (vol/vol) Triton X-100 for 10 min on ice. The cells were washed with PBS, and nonspecific binding was blocked by incubation in 10% bovine serum in PBS for 1 h at room temperature. Then the cells were incubated with an anti-YY1 antibody (Santa Cruz, sc-1703) or an anti-GATA3 antibody (Santa Cruz, sc-268) in 10% (vol/vol) bovine serum in PBS for 2 h at room temperature.

For the immunofluorescence assay, Alexa Flour 647–conjugated anti-mouse IgG (Invitrogen A21236) or Alexa Flour 546-conjugated anti-rabbit IgG (Invitrogen A11035) in PBS with 10% bovine serum was added and incubated for 1 h at room temperature. After the cells were washed extensively with PBS (10% buffered saline), DNA was counterstained with Vectashield Mounting Medium with DAPI (Vector). Images were recorded with an Olympus BX53 fluorescence microscope.

For the in situ proximity ligation assay, Duolink (Olink Biosciences) in situ proximity ligation assay was performed according to the manufacturer's protocol. Proximity ligation assay probes were

diluted in 10% bovine serum in PBS, and incubated in a preheated humidity chamber for 1 h at 37 °C, followed by hybridization, ligation, amplification, and detection. After the cells were washed, DNA was counterstained with DAPI (Vector). Images were recorded with an Olympus BX53 fluorescence microscope.

Animal Model of Allergic Asthma. Experimental methods for sensitization and challenge with ovalbumin, total and differential cell counting of bronchoalveolar lavage fluid, lung histology, and measurement of serum IgE were described previously (1).

1. Koh BH, et al. (2010) Th2 LCR is essential for regulation of Th2 cytokine genes and for pathogenesis of allergic asthma. *Proc Natl Acad Sci USA* 107(23):10614–10619.
2. Kim J, Kim JD (2008) In vivo YY1 knockdown effects on genomic imprinting. *Hum Mol Genet* 17(3):391–401.
3. Amsen D, et al. (2007) Direct regulation of Gata3 expression determines the T helper differentiation potential of Notch. *Immunity* 27(1):89–99.
4. Hwang SS, Lee S, Lee W, Lee GR (2010) GATA-binding protein-3 regulates T helper type 2 cytokine and ifng loci through interaction with metastasis-associated protein 2. *Immunology* 131(1):50–58.
5. Spilianakis CG, Flavell RA (2004) Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. *Nat Immunol* 5(10):1017–1027.

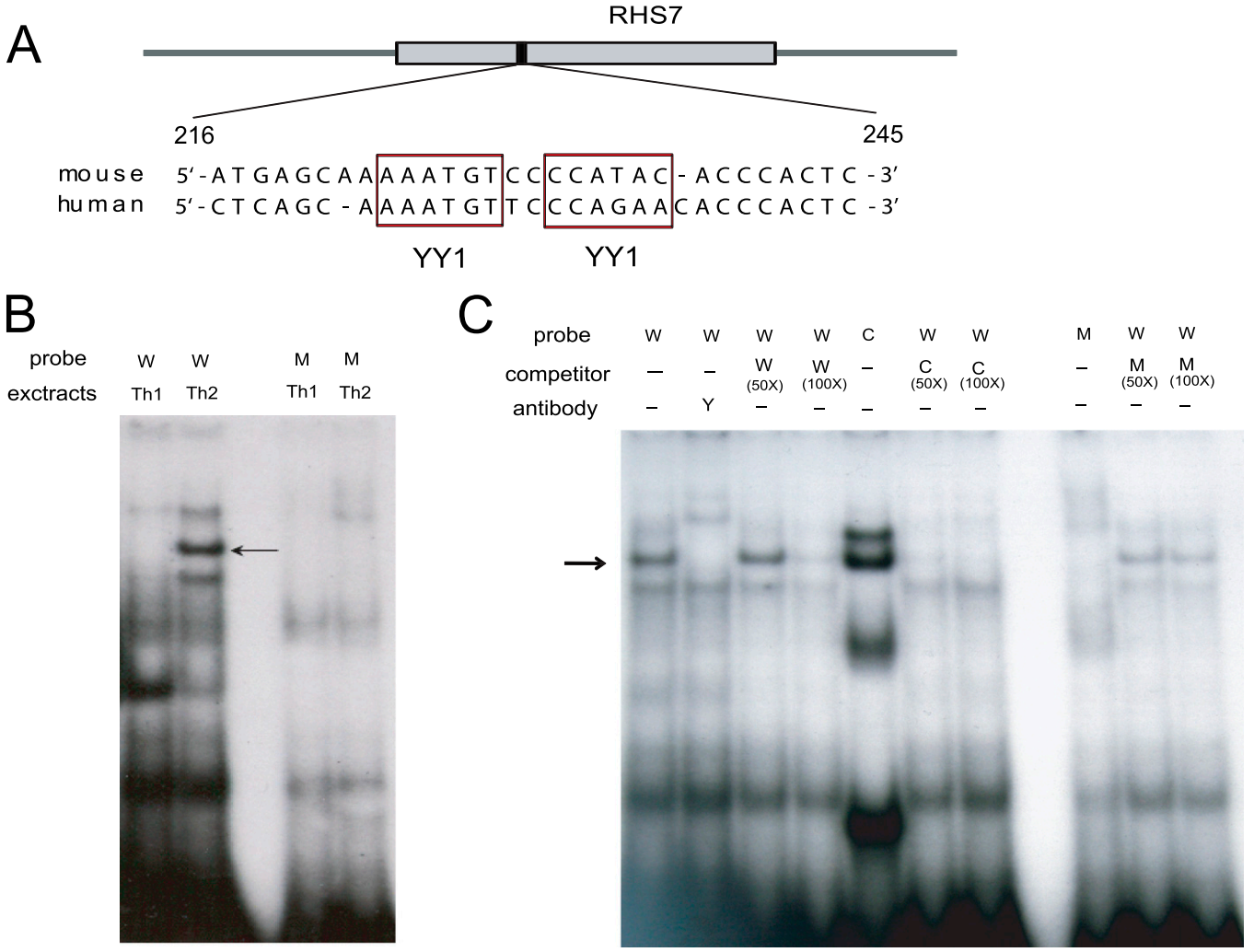

Fig. S1. YY1 binding to RHS7. (*A*) Position and DNA sequence of RHS7b. RHS7 position is 55649–56285 bp downstream from the *rad50* translation start site. RHS7b position is 216–245 within RHS7. Two inverted YY1 binding sites are shown. (*B*) EMSA with RHS7b and Th1 or Th2 cell extracts. The arrow indicates the YY1 band. (*C*) Supershift assay with an anti-YY1 antibody or competition assay with competitor oligonucleotides nucleotides. C, consensus YY1 binding site oligonucleotide; M, RHS7b-mutant oligonucleotide; W, RHS7b oligonucleotide; Y, anti-YY1 antibody. The arrow indicates the YY1 band.

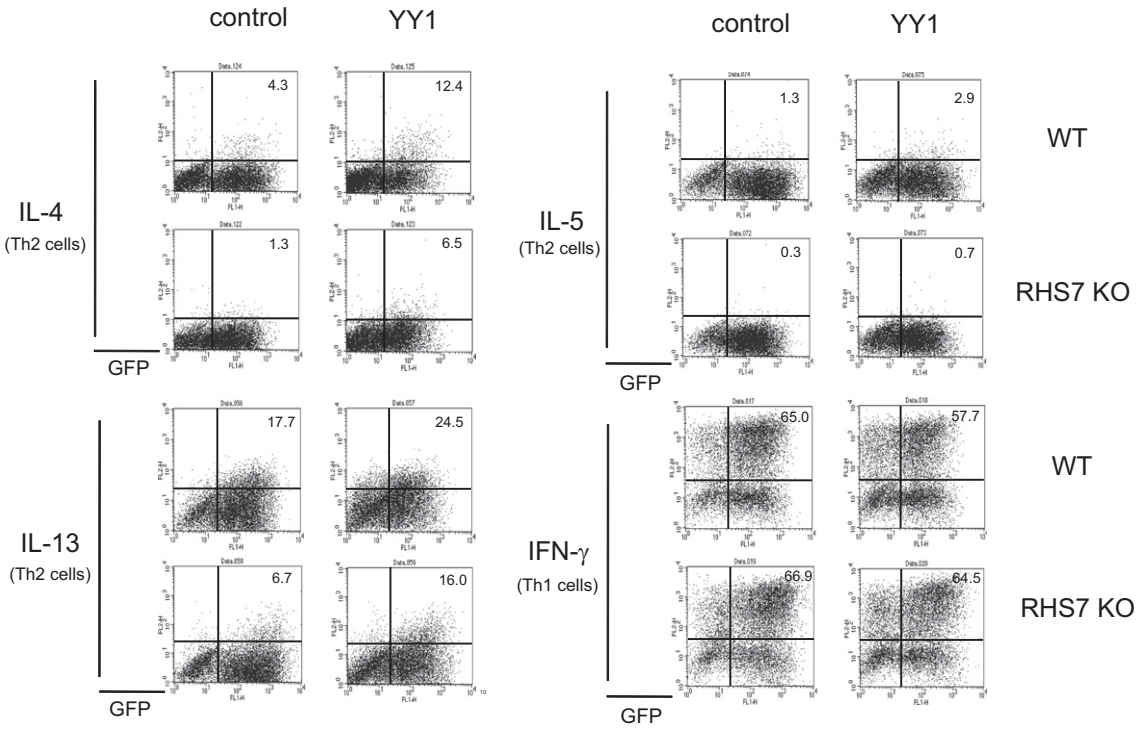

Fig. S2. The effect of YY1 overexpression on the expression of cytokines in Th1 or Th2 cells. Naive CD4 T cells were transduced with *yy1*-retroviral vector or control MIEG3 retroviral vector and induced to differentiate into Th1 or Th2 cells. Cytokine expression was measured by ICS using FACS. Numbers in the plots show percentage of cells from GFP^+ cells. Data are representative of three independent experiments with similar results.

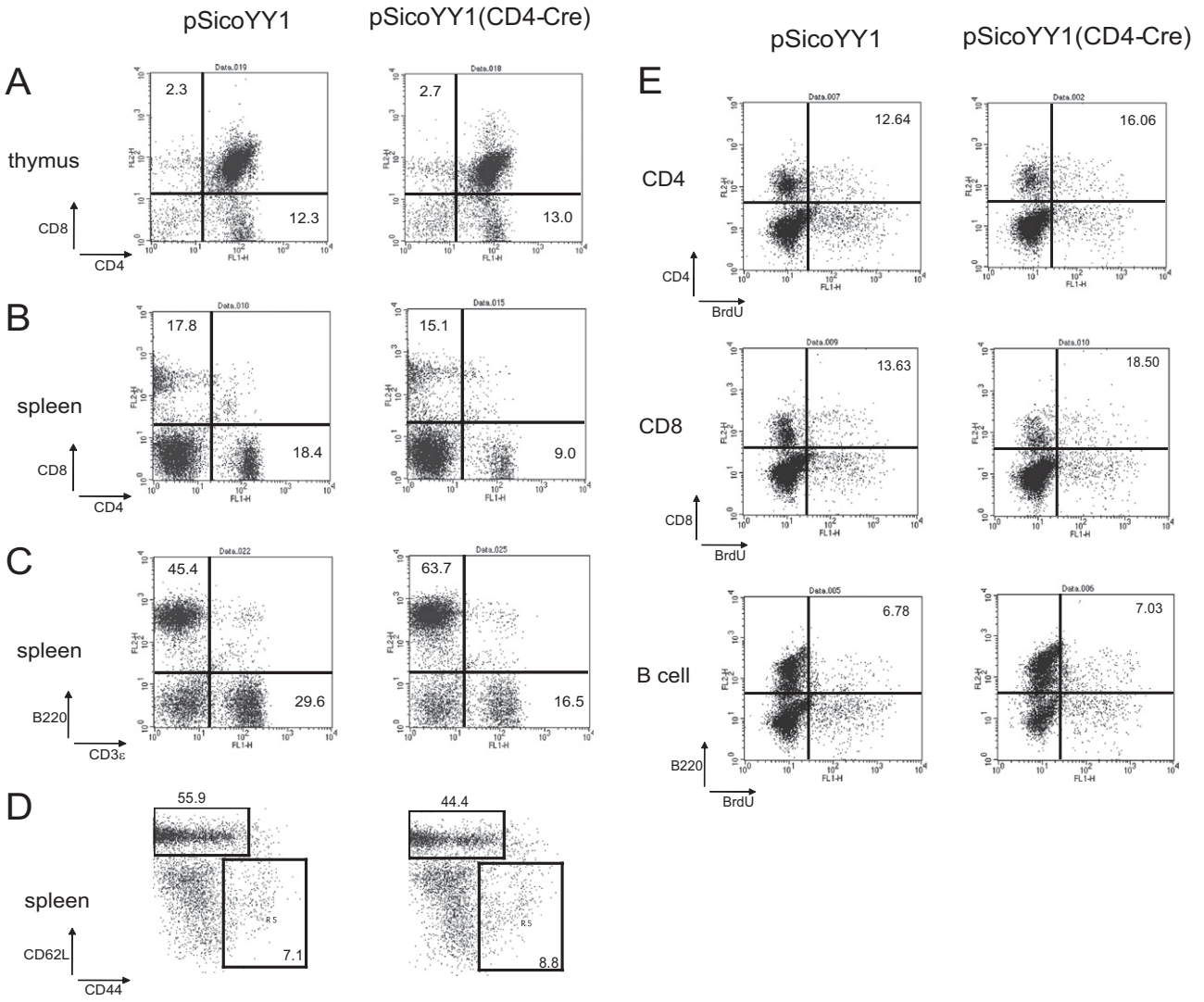

Fig. S3. Ratios of cell populations (*A*–*D*) and proliferation capacity of CD4, CD8, and B cells (*E*) from pSicoYY1 and pSico(CD4-Cre) mice. (*A*) Ratios of CD4 to CD8 T cells in the thymus. (*B*) Ratios of CD4 to CD8 T cells in the spleen. (*C*) Ratios of T to B cells in the spleen. (*D*) Ratios of naive to effector/memory CD4 T cells in the spleen. Cells were gated on CD4 T cells. (*E*) Splenocytes were cultured in the presence of 1 mM BrdU (BD) and 1 ng/mL of IL-2 for 18 h. The incorporation of BrdU into DNA was measured by staining with a FITC-conjugated anti-BrdU antibody.

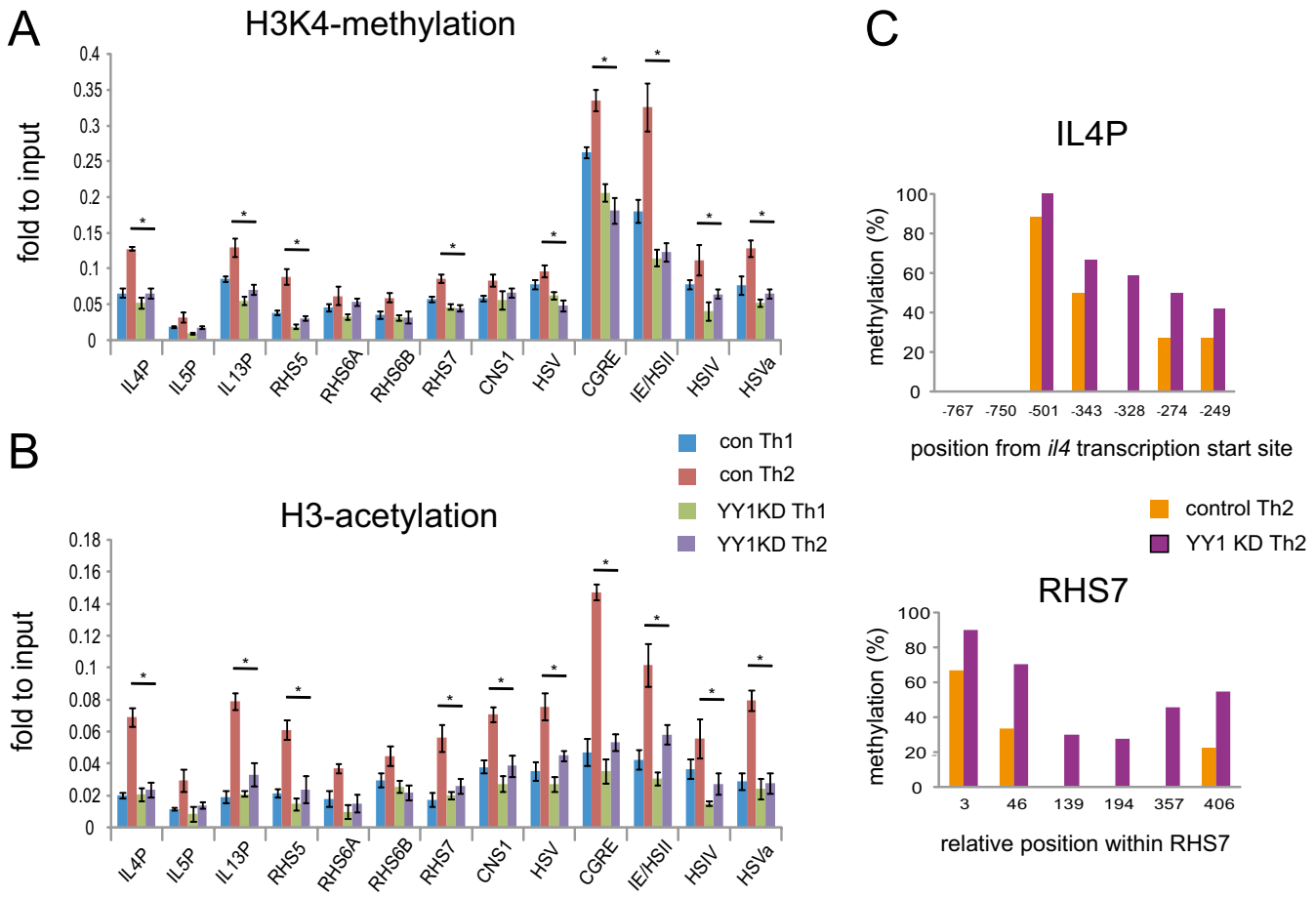

Fig. S4. Repressed histone marks (*A* and *B*) and increased DNA methylation (*C*) in YY1 KD Th2 cells. Naive CD4 T cells were isolated from control (pSico-YY1) or YY1 KD [pSico-YY1(CD4-Cre)] mice, and differentiated under a Th1 or Th2 polarizing condition. H3K4-methylation (*A*) or H3-acetylation (*B*) in regulatory elements in the Th2 cytokine locus was measured by ChIP using a mixture of anti-trimethyl-H3K4 and anti-monomethyl H3K4 antibodies, an anti-acetyl H3 antibody, or a control IgG antibody. The value of control IgG antibody was subtracted in the graph. Statistical difference between control Th2 and YY1KD Th2 was analyzed by Student *t* test. Data are representative of two independent experiments with similar results. (*C*) DNA methylation in Th2 cells was measured by a bisulfite modification method as described in *SI Materials and Methods*. Each bar represents a percentage of methylation of 15–22 independent fragments. RHS7 position is 55649–56285 bp downstream from the *rad50* translation start site.

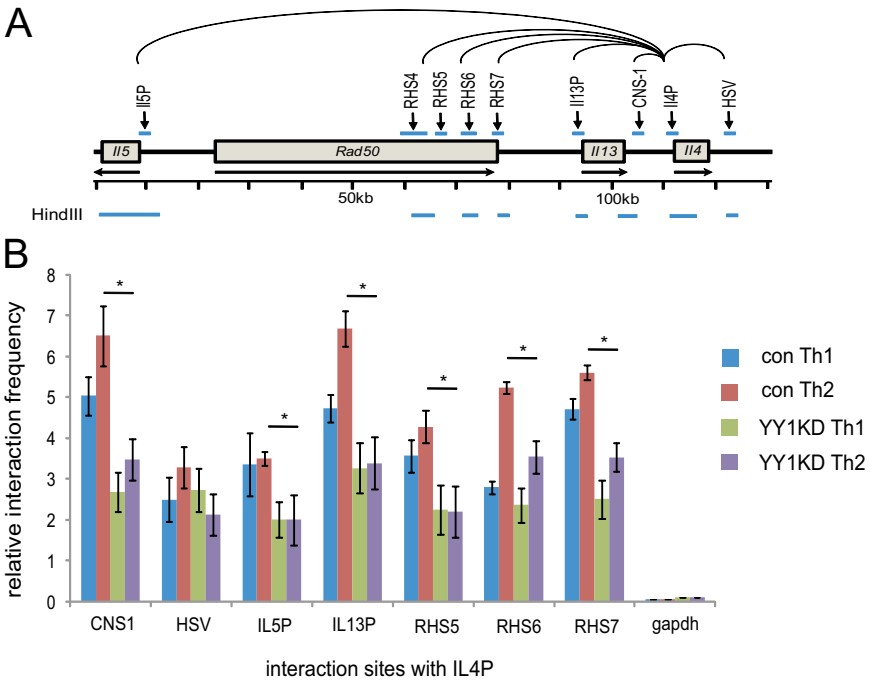

Fig. S5. Reduced long-range chromosomal interactions in YY1 KD Th2 cells. (*A*) Schematic of the Th2 cytokine locus showing HindIII digestion fragments and their interactions measured in the experiment. (*B*) Cells were differentiated as described in Fig. 4. Long-range chromosomal interactions between the *il4* promoter and other fragments in the Th2 cytokine locus were measured by 3C. Statistical difference between control Th2 and YY1KD Th2 was analyzed by Student *t* test. $n = 3$. $^*P < 0.05$. Data are representative of three independent experiments with similar results.

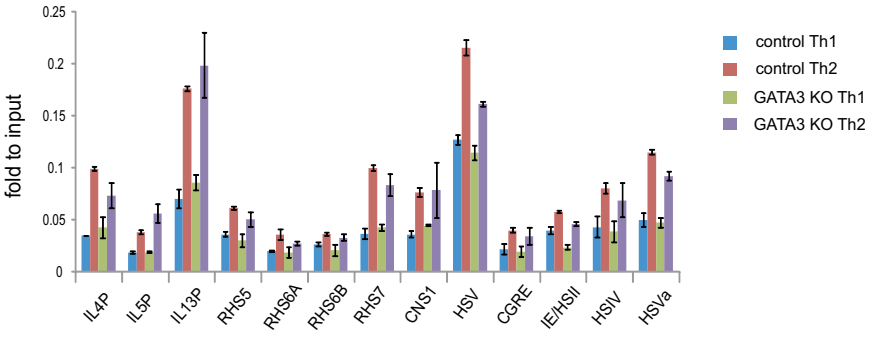

Fig. S6. YY1 binding to the Th2 cytokine locus in GATA3 KO Th2 cells. Naive CD4 T cells from control or GATA3$^{f//fl}$ mice were transduced with control or *Cre*-retroviral vectors and induced to differentiate into Th1 or Th2 cells. YY1 binding to regulatory elements in the Th2 cytokine locus was measured by ChIP using an anti-YY1 antibody. Data are representative of three independent experiments with similar results.

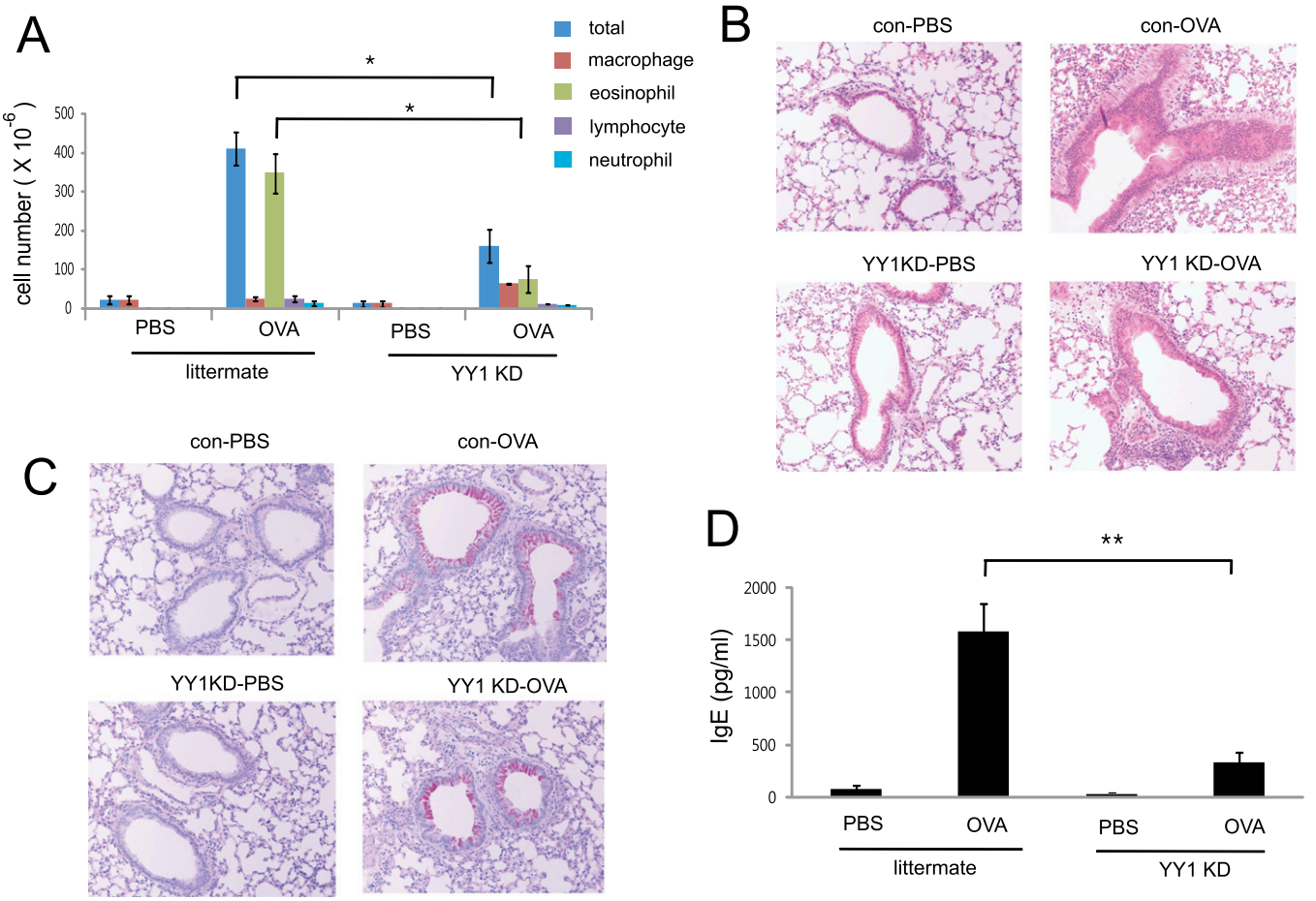

Fig. S7. Animal model of allergic asthma. Control and YY1 KD mice were sensitized and challenged with OVA. (*A*) Total and differential cell numbers were counted in bronchoalveolar lavage fluid by Diff-Quik staining. Statistical differences of total numbers and eosinophil numbers between littermate control and YY1 KD were analyzed by Student *t* test. $n = 3$. Data are representative of three independent experiments with similar results. (*B*) Lung sections were stained by hematoxylin and eosin. (*C*) Lung sections were stained with PAS. (*D*) The relative amount of serum IgE was measured by ELISA. Statistical difference between littermate control and YY1 KD was analyzed by Student *t* test. $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$.

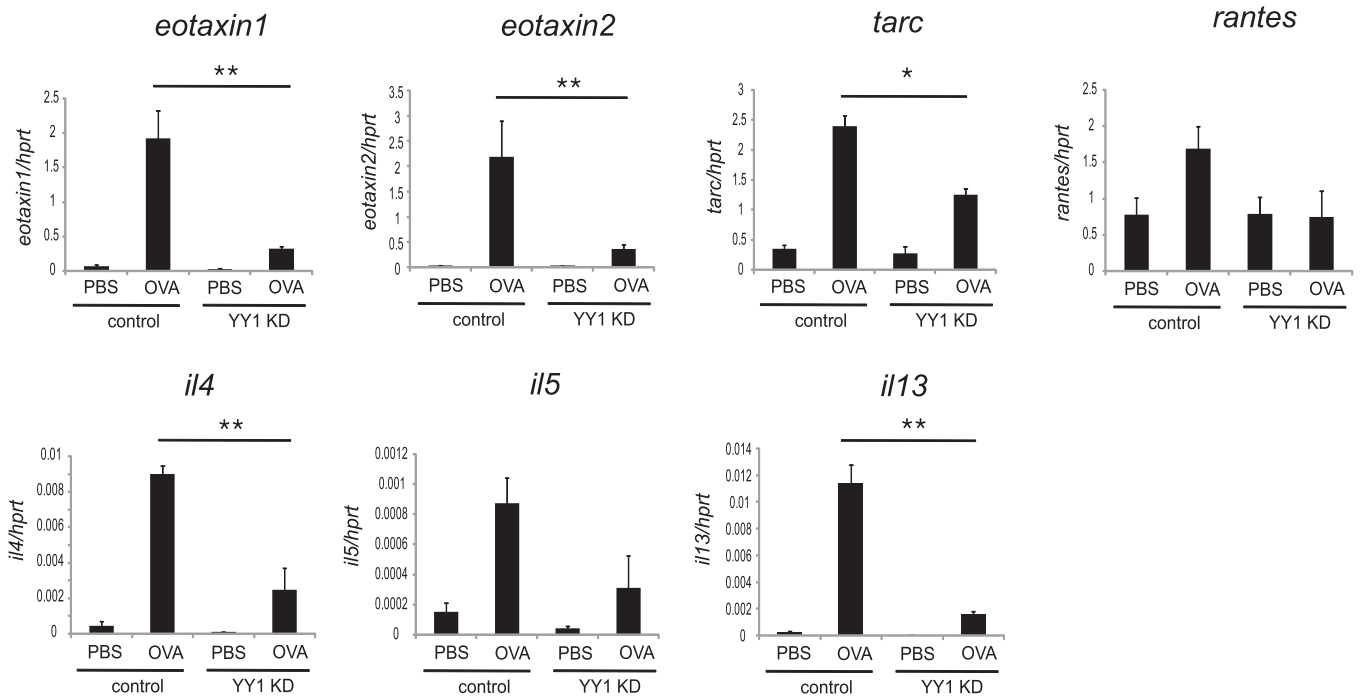

Fig. S8. Cytokine and chemokine gene expression in control and YY1 KD mice. Mice were sensitized and challenged with OVA or control PBS as described in the *Materials and Methods* section. Total RNA from the lung of the mice was isolated, and the expression of chemokine and cytokine genes was analyzed by quantitative RT-PCR. Error bars indicate SD (n = 3). Statistical difference between control Th2 and YY1KD Th2 was analyzed by Student t test. n = 3. *$P < 0.05$, **$P < 0.01$. Data represent two independent experiments with similar results.

Table S1. Primers for chromatin immunoprecipitation

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
RHS7 - Fw	TGC CTG CCC TGC AAA CA
RHS7 - Rv	TGT GGG AGG AGA TAG GAC TCT TAC C
RHS6b - Fw	CAC CTT TGT CAC TTA AAC ACA TCG T
RHS6b - Rv	GCA GCC AGT GCT CCT AAG GA
RHS6a Fw	GGC TCC CTC TGC TCT GAC T
RHS6a Rv	TAA AAA GGG AAA ATG GTA ACA AAA
RHS5 Fw	AAT TTC CCT CCT TGT TTG TCG T
RHS5 Rv	CTG CTC ACT GCG CTT TAG ATG
HSVa Fw	CCC TCC ATA CAG TTC CTG CAG TA
HSVa Rv	ACC AGG GCA CTT AAA CAT TGC T
HSIV Fw	CTG CAT ACC TTC CCT GAT TGG
HSIV Rv	CCC TGC CCA CAT GAA ATA CC
HSII Fw	AGGCCTCTTTCTGGTCACCTAACA
HSII Rv	GTCCCCTCCCACTACAAATGGA
CGRE Fw	GTCCTCTTATCGACCCCATC
CGRE Rv	AAAGGCTTGGGGAAACAC

Table S2. Primers for quantitative RT-PCR

Primer name	Sequence
il4 sense	5′-AGATCATCGGCATTTTGAACG-3′
il4 anti-sense	5′-TTTGGCACATCCATCTCCG-3′
*il4*probe	(FAM)-5′-TCACAGGAGAAGGGACGCCATGC-3′-(Tamra)
ifng sense	5′-GGATGCATTCATGAGTATTGC-3′
ifng anti-sense	5′-CCTTTTCCGCTTCCTGAGG-3′
ifng probe	(FAM)-5′-TTTGAGGTCAACAACCCACAGGTCCA-3′-(Tamra)
hprt sense	5′-CTGGTGAAAAGGACCTCTCG-3′
hprt anti-sense	5′-TGAAGTACTCATTATAGTCAAGGGCA-3′
hprt probe	(FAM)-5′-TGTTGGATACAGGCCAGACTTTGTTGGAT-3′(Tamra)
il5 sense	5′-CGCTCACCGAGCTCTGTTG-3′
il5 anti-sense	5′-CCAATGCATAGCTGGTGATTTTT-3′
il5 probe	FAM-5′-CAATGAGACGATGAGGCTTCCTGTCCC-3′-Tamra
il13 sense	5′-GCTTATTGAGGAGCTGAGCAACA-3′
il13 anti-sense	5′-GGCCAGGTCCACACTCCATA-3′
il13 probe	FAM-5′-CAAGACCAGACTCCCCTGTGCAACG-3′-Tamra
gata3 sense	5′-AGAACCGGCCCCTTATCAA-3′
gata3 anti-sense	5′-AGTTCGCGCAGGATGTCC-3′
gata3 probe	FAM-5′-CCAAGCGAAGGCTGTCGGCAG-3′-Tamra
tbx21 sense	5′-CAACAACCCCTTTGCCAAAG-3′
tbx21 anti-sense	5′-TCCCCCAAGCAGTTGACAGT-3′
tbx21 probe	FAM-5′-CCGGGAGAACTTTGAGTCCATGTACGC-3′-Tamra
actb F	CGTGAAAAGATGACCCAGATCA
actb R	TGGTACGACCAGAGGCATACAG
actb probe	FAM TCAACACCCCAGCCATGTACGTAGCC Tamra

Table S3. Primers for the 3C assay

Primer name	Sequence
IL4P-CNS1F	CTTGGGCCAATGAGATGG
IL4P-CNS1 R	TTCTTGGGGCCTGGAGAG
IL4P-CNS2 F	CTTGGGCCAATGAGATGG
IL4P-CNS2 R	TTCTTGGGGCCTGGAGAG
IL4P-IL5P F	ATTCTTGGGCCAATGAGATG
IL4P-IL5P R	CAGGTCCTGCCAGAGCTAA
IL4P-IL13P F	ATTCTTGGGCCAATGAGATG
IL4P-IL13P R	GCAGGGGAAATGCCTTATC
IL4P-RHS45 F	ATTCTTGGGCCAATGAGATG
IL4P-RHS45 R	GAACAAACCCGAAAGAGACAA
IL4P-RHS6 F	CTTGGGCCAATGAGATGG
IL4P-RHS6 R	TGAAAGAAGAAATGCTTGTGAA
IL4P-RHS7 F	CTTGGGCCAATGAGATGG
IL4P-RHS7 R	ATGTGACAGCAGGGCTACG
IL4P probe (Th2 locus)	AGGAGGGAAGGTGCTTGCCG
IL4P-GAPDH F	CTTGGGCCAATGAGATGG
IL4P-GAPDH R	GCAAACCTCCTCCAAGTCAT
IL4P-GAPDH probe	AGGAGGGAAGGTGCTTGCCG