Supporting Information

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SI Materials and Methods

In Vitro Treg Assay. CD4$^+$CD45RB^{lo}CD25$^+$ cells were sorted by FACS Aria and used as regulatory T cells (Treg). CD4$^+$CD45RB^{hi}CD25$^-$ cells were sorted and labeled with 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) and used as conventional T cells (Tconv). Tconv cells (2.5×10^4 cells per well) were mixed with Treg cells at ratios indicated in Fig. S2*F*, and stimulated with 6.3×10^4 per well of Dynabeads mouse T-Activator CD3/CD28 (Invitrogen) in a 96-well round bottom plate. After 3 d of culture, the cells were analyzed by flow cytometer, and the proliferation was assessed by CFSE dilution.

Colitis Model. Colitis was induced in Rag1$^{-/-}$ mice by i.p. injection of 5×10^5 of CD4$^+$CD45RB^{hi}CD25$^-$ T cells. Mice were monitored for weight loss and were killed after 9 wk. For histological analysis, frozen large intestine sections were stained with hematoxylin and eosin.

***Listeria monocytogenes* Infection.** Control or Bach2 flox/flox × lck-cre mice were infected with 1.5×10^4 of *L. monocytogenes* (EGD strain) intraperitoneally. Mice were analyzed 7 d after infection.
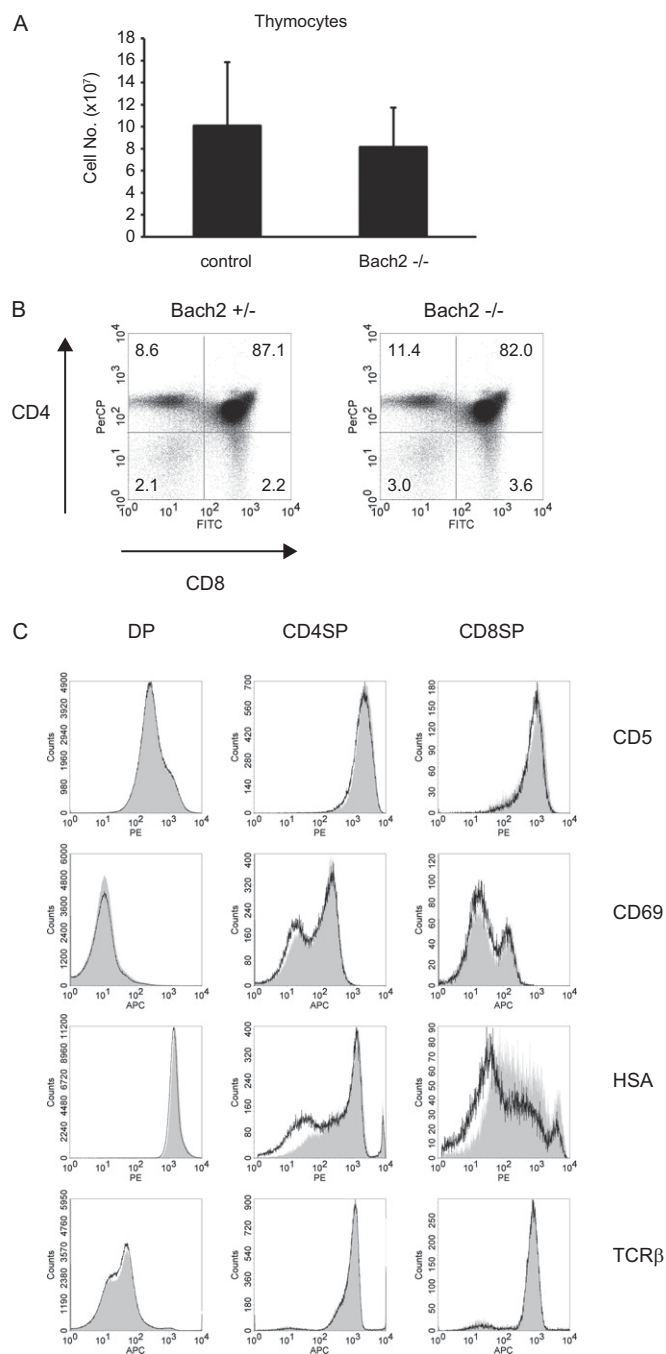
Fig. S1. Thymocyte differentiation in Bach2$^{-/-}$ mice. (*A*) Cell numbers of thymocytes (mean ± SD, *n* = 7). (*B*) Thymocyte subpopulations by the CD4/CD8 expression in Bach2$^{-/-}$ mice. (*C*) Expression of differentiation markers in each thymocyte subpopulation. The open and filled gray histograms show Bach2$^{-/-}$ and $^{+/-}$ cells, respectively. APC, allophycocyanin; CD4SP, CD4 single positive; DP, double positive; HSA, heat stable antigen; PE, phycoerythrin; perCP, peridinin chlorophyll protein.
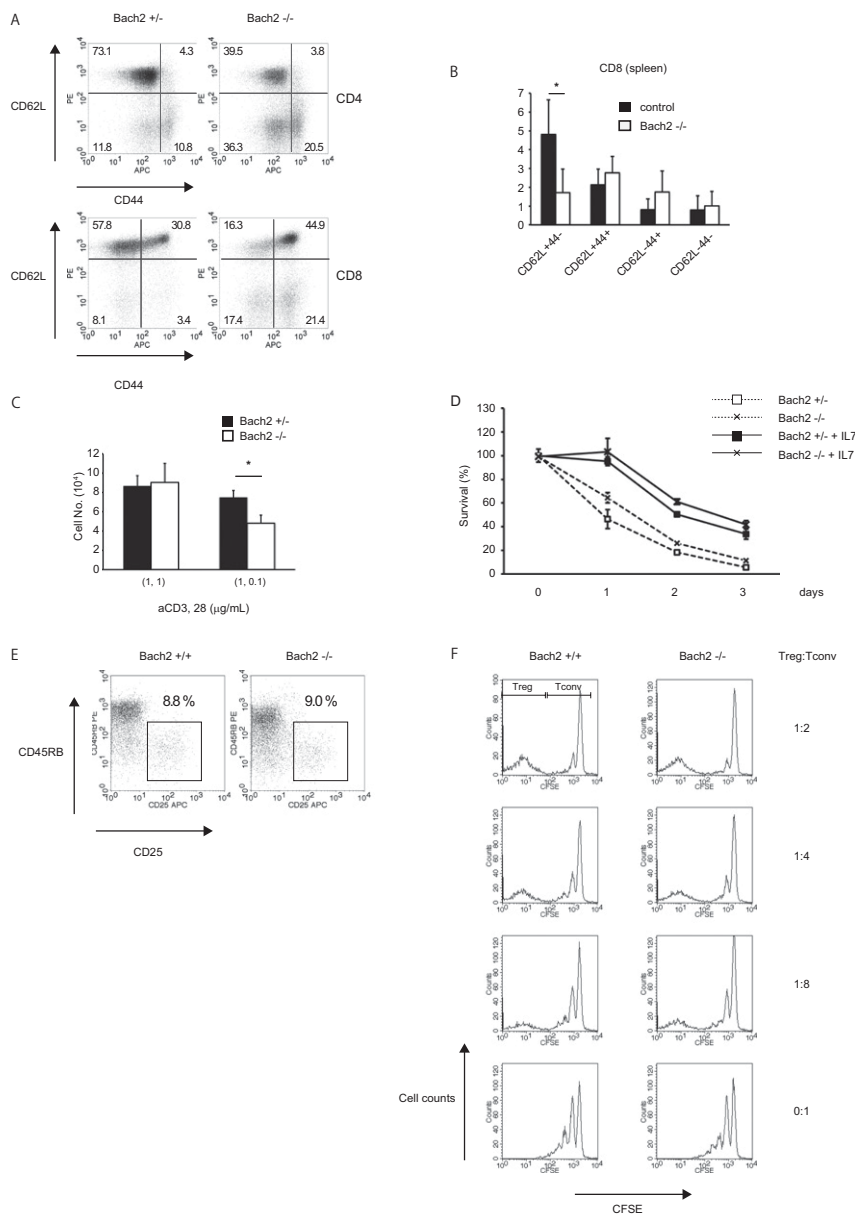
Fig. S2. Effects of Bach2 deficiency on mature peripheral T cells. (*A* and *B*) Naive and memory T cells in the spleen of Bach2$^{+/-}$ and $^{-/-}$ mice. FACS profiles (*A*) and the cellularity of each population (*B*) are shown. (*C*) Proliferation of peripheral T cells after TCR stimulation in vitro. Naive CD4 T cells were stimulated with the indicated amount of the plate-coated anti-CD3/CD28 Abs. (*D*) Survival capacities of Bach2 $^{+/-}$ and Bach2$^{-/-}$ naive CD4 T cells. Cells were culture in the presence or absence of IL7 without TCR stimulation. Cell numbers were shown as the percentages to those at day 0. (*E*) No difference was observed in the percentage of regulatory T cells (CD45RB^{lo}CD25^{+}, Treg) in CD4^{+} splenocytes. Control or Bach2$^{-/-}$ splenocytes were stained with anti-CD4, CD45RB, and CD25 antibodies. The cells were analyzed by flow cytometer. The figure shows the expression of CD45RB and CD25 in CD4^{+}-gated cells. The numbers in the panels indicate the percentages of Treg. (*F*) In vitro Treg assay did not show significant difference in suppressive activity of control and Bach2$^{-/-}$ Treg. Control or Bach2$^{-/-}$ Treg cells were sorted and mixed with CFSE-labeled wild-type conventional naive CD4^{+} cells at the indicated ratios. The cells were stimulated with anti-CD3/28 beads for 3 d, and the proliferation of the cells was assessed by the dilution of CFSE. Data are expressed as mean $\pm$ SD, $n = 3$, *$P < 0.05$ in *A*–*D*.
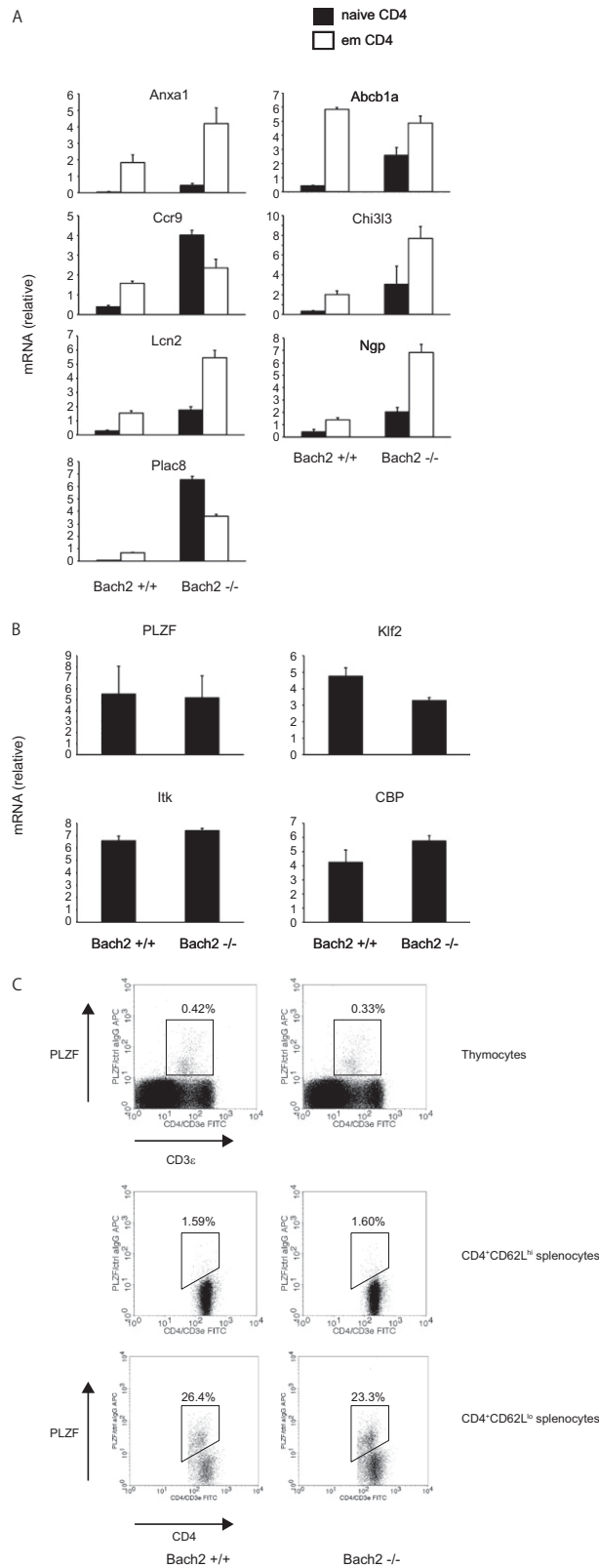
Fig. S3. Bach2 suppresses effector memory-related genes in naive T cells. (*A*) Altered gene expression profiles by Bach2 deficiency. mRNA expression of the genes other than the ones in Fig. 2 that are shown to be up-regulated in Bach2-deficient unstimulated and stimulated naive CD4 T cells in the microarray were examined by quantitative RT-PCR (qPCR) for naive and effector-memory CD4 T cells. (*B*) No alteration of the expressions of promyelocytic leukemia zinc finger protein (PLZF), IL2-inducible T-cell kinase (Itk), kruppel-like factor 2 (Klf2), and cAMP response element binding protein-binding protein (CBP) by Bach2 deficiency in T cells. The expressions of each gene in naive CD4 T cells were assessed by qPCR (*C*) No significant difference was observed in PLZF protein expression of T-cell populations. Indicated T-cell populations were permeabilized and stained with anti-PLZF antibody and then analyzed by flow cytometer. The numbers in each panel are the percentages of PLZF$^+$ cells in each population. Data are expressed as mean $\pm$ SD, $n = 3$ in *A* and *B*. Abcb1a, ATP-binding cassette, sub-

family B (MDR/TAP), member 1A; aIgG, anti-IgG Ab; Anxa1, annexin A1; Ccr9, CC chemokine receptor 9; Chi3l3, chitinase 3-like 3; em, effector-memory; Lcn2, lipocalin 2; Ngp, neutrophilic granule protein; Plac8, placenta-specific 8.
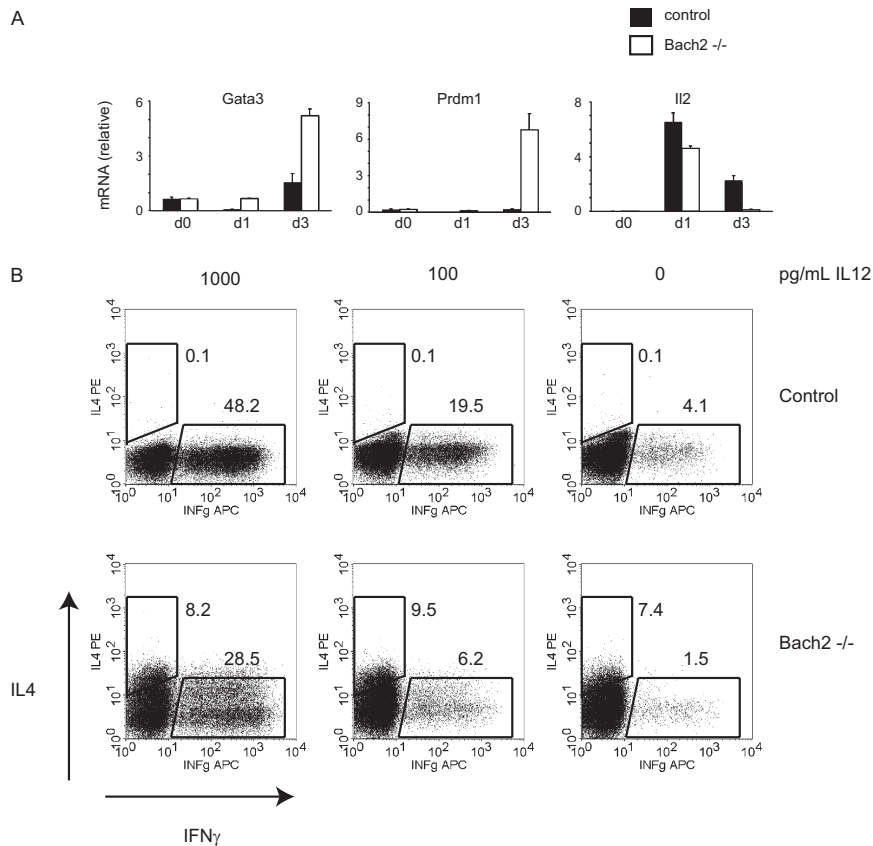
Fig. S4. Bach2-deficiency promotes Th2 differentiation. (*A*) Up-regulation of Th2-related genes GATA binding protein 3 (Gata3) and PR domain zinc finger protein 1 (Prdm1) in Bach2$^{-/-}$ CD4 T cells in addition to the ones in Fig. 6*A* whereas IL2 expression was decreased at day 3. mRNA expression of naive CD4 T cells was analyzed at day 0, 1, and 3 after simulation with anti-CD3 and CD28 Abs. Data are expressed as mean ± SD, *n* = 3. (*B*) Th differentiation of Bach2$^{-/-}$ CD4 T cells in the presence of IL-12. CD4 naive T cells were stimulated with anti-CD4 and CD28 Abs with or without graded amounts of IL-12. After 5 d, the cells were restimulated with phorbol-12-myristate-13-acetate (PMA) plus ionomycin in the presence of brefeldin A for 4 h. The intracellular IFNγ and IL-4 were stained and analyzed by flow cytometer.
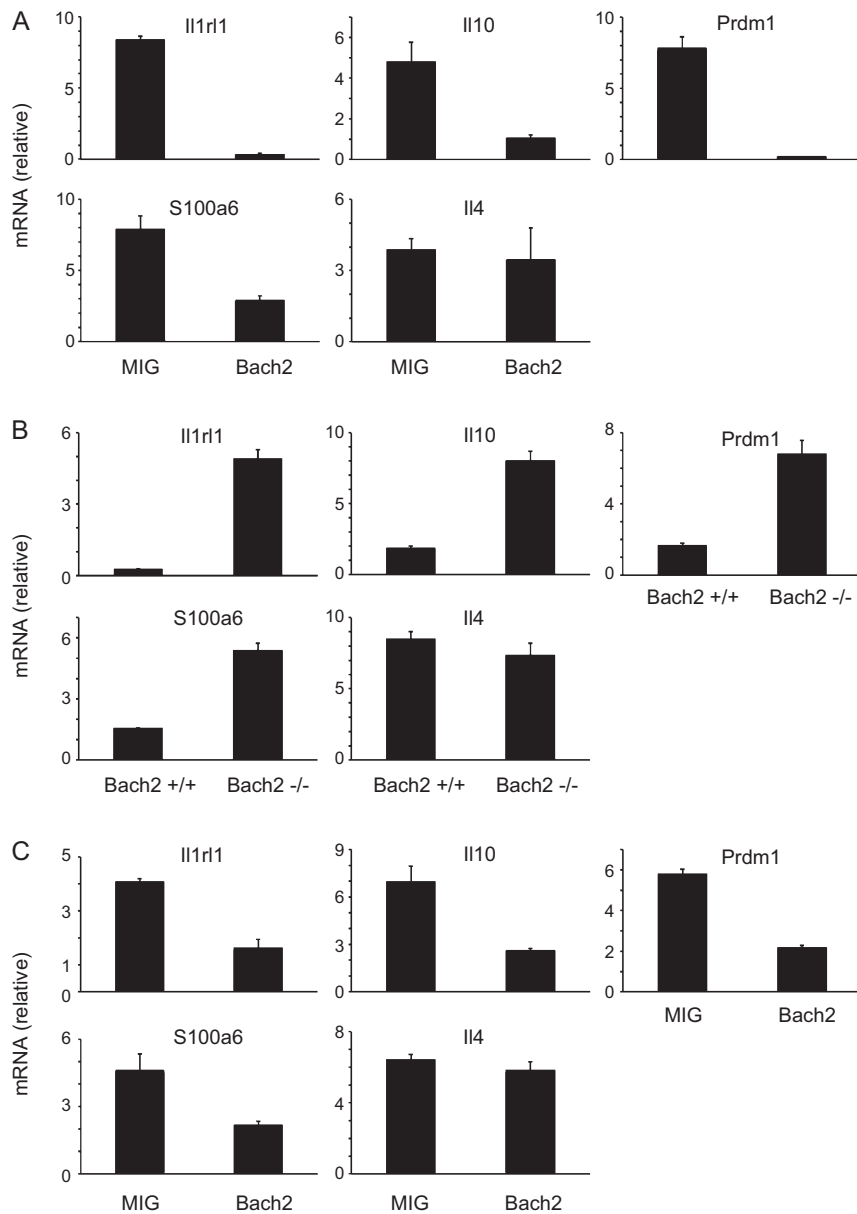
Fig. S5. Suppression of genes expression that are up-regulated in Bach2$^{-/-}$ T cells by Bach2 overexpression. (*A*) Bach2$^{-/-}$ naive CD4 T cells were stimulated and transduced by retrovirus-mediated transfection of Bach2 or control vector (MIG). On day 3 after transfection, mRNA was purified from and assessed by qPCR for each gene. (*B*) Control and Bach2$^{-/-}$ naive CD4+ T cells were stimulated at the same condition of *A* and *B*, and their mRNA expressions of the indicated genes were analyzed by qPCR. (*C*) WT effector-memory (CD62L^{lo} CD44^{hi}) CD4 T cells were stimulated and transduced with Bach2 or control vector (MIG), and mRNA expressions were assessed. Data are expressed as mean ± SD, *n* = 3. Il1rl1, interleukin-1 receptor-like 1; S100a6, S100 calcium binding protein a6.
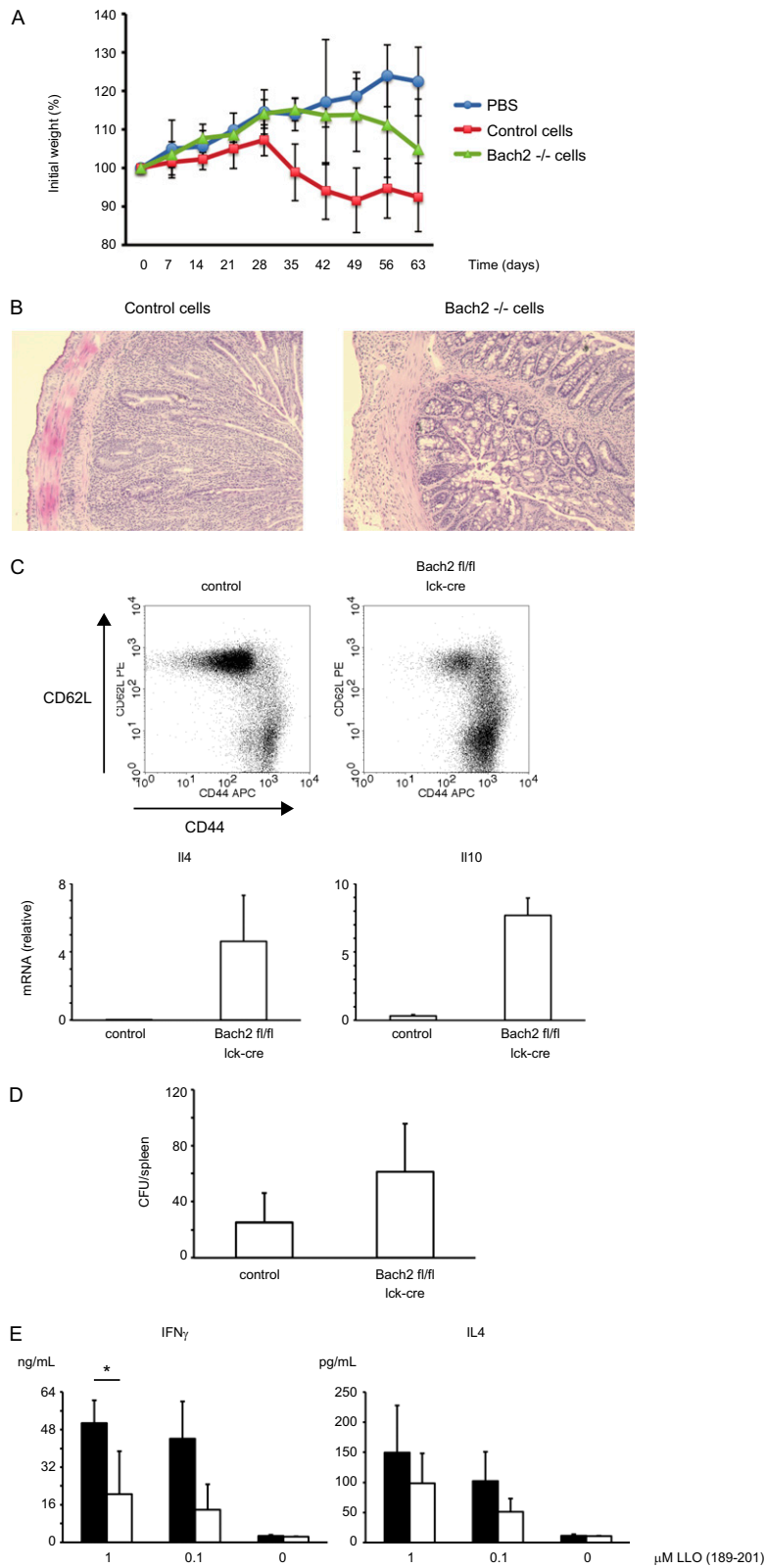
Fig. S6. The effects of Bach2 deficiency on in vivo immune responses. (*A* and *B*) The effects on colitis induced by transfer of naive CD4 T cells. (*A*) Body weight of Rag1-deficient mice after the transfer of CD4$^+$CD25$^-$CD45RB^{hi} naive T cells. Data are presented relative to initial body weight and as mean ± SD (*n* = 3–7). (*B*) Hematoxylin and eosin staining of colon sections from Rag1-deficient mice at 9 wk after the cell transfer as in *A*. (*C–E*) The effects on *L. monocytogenes* (LM) infection. Control or Bach2 conditional knockout (cKO) mice [Bach2 flox/flox (fl/fl) crossed with lck-cre] were infected with 1.5 × 10^4 LM intraperitoneally. Mice were analyzed 7 d after infection. (*C*) Bach2 cKO shows similar phenotypes to Bach2$^{-/-}$ CD4 T cells. (*Upper*) CD62L and CD44 expression in CD4$^+$ cells of control or Bach2fl/fl x lck-cre mice. (*Lower*) mRNA expression of each gene in control or Bach2 fl/fl x lck-cre naive CD4 T cells at day 3 after TCR simulation (anti-CD3 and CD28 mAbs). Data are expressed as mean ± SD, *n* = 3. (*D*) Numbers of viable LM in spleen are shown. CFU, colony forming unit. Data are expressed as mean ±
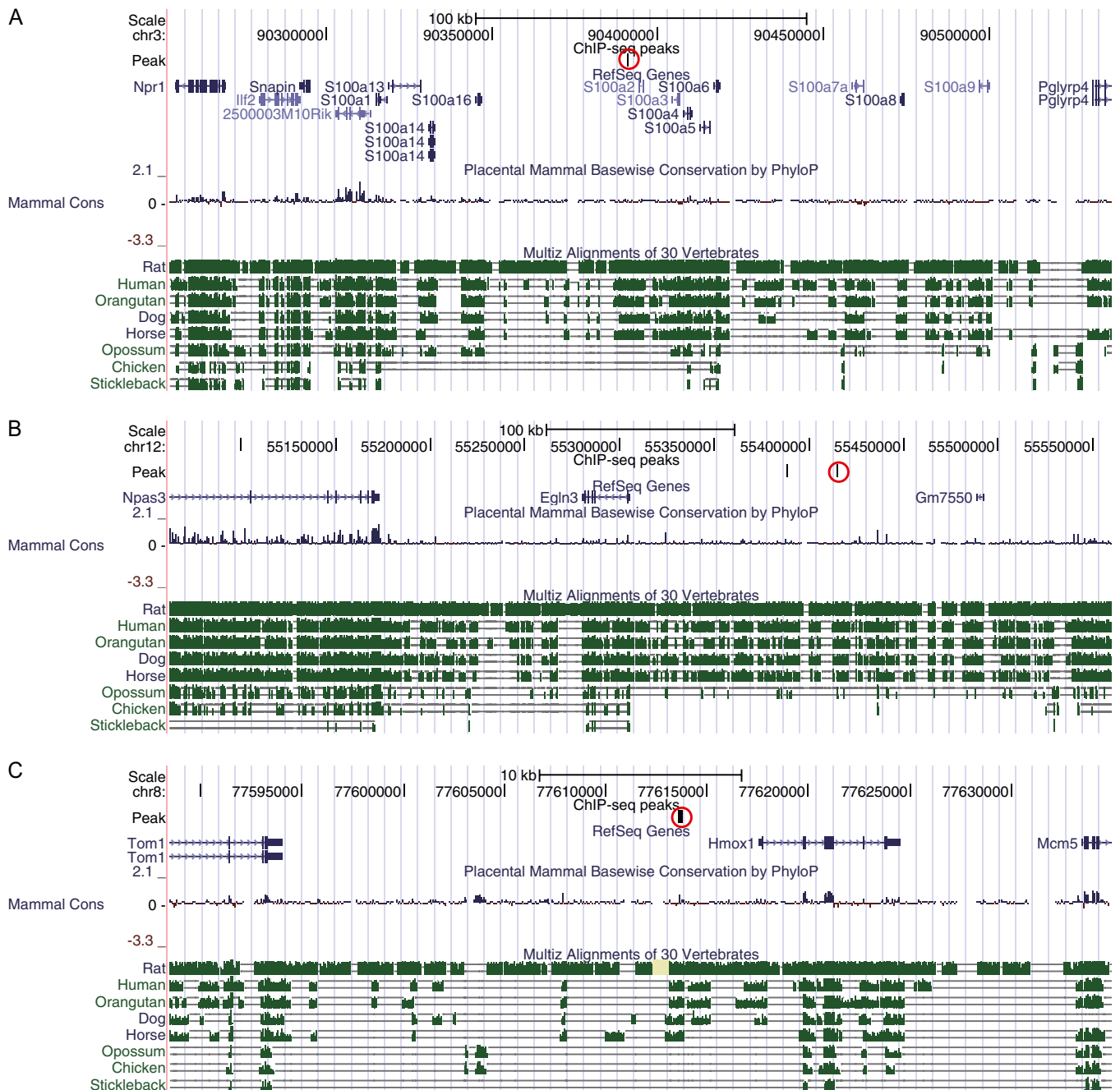
Fig. S7. The genomic loci selected by Chromatin Immunoprecipitation (ChIP)-seq for FLAG-Bach2C. Screen shots from the UCSC Genome Browser (University of California, Santa Cruz) with ChIP-seq peaks (red circle) are shown. The scale is indicated in each graph. The peaks around the S100a gene cluster (*A*), egl nine homolog 3 [Egln3, encoding prolyl hydroxylase 3 (PHD3)] (*B*), and heme oxygenase 1 [Hmox1, encoding heme oxigenase 1 (HO-1)] (*C*) are shown. Npr1, natriuretic peptide receptor 1; Snapin, SNAP-associated protein; Pglyrp4, peptidoglycan recognition protein 4; chr, chromosome; Npas3, neuronal PAS domain protein 3; Tom1, target of myb1 homolog; Mcm5, minichromosome maintenance deficient 5.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XSLX)