Figure S1

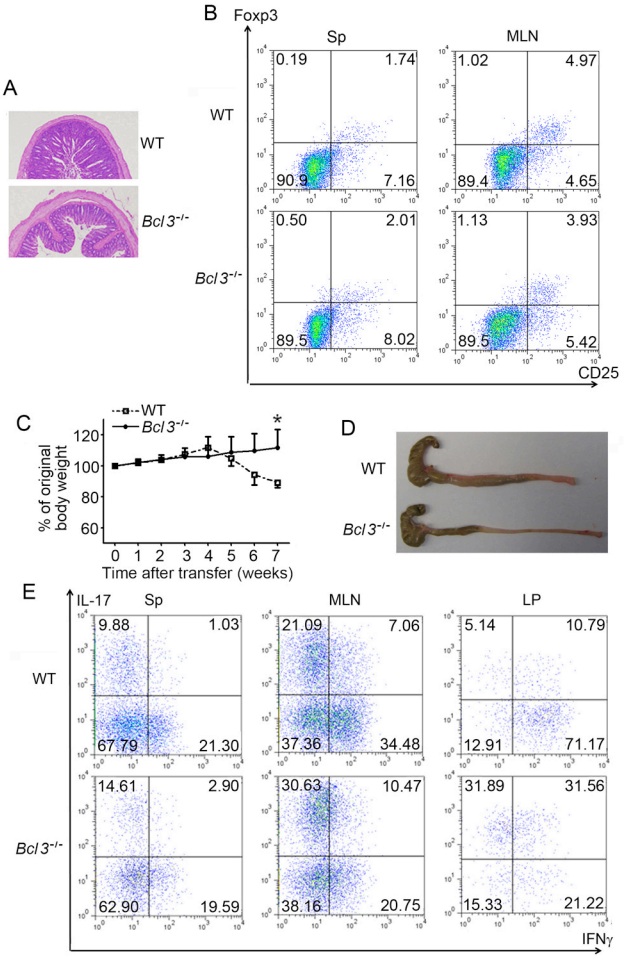

Figure S1. Loss of Bcl-3 in T cells protects against T cell transfer-induced colitis and is associated with a shift from Th1 to Th17. (A) $CD4^+$ $CD45RB^{hi}$ $CD25^-$ naïve wild-type (WT) and *Bcl3*$^{-/-}$ T cells were injected i.p. into *Rag1*$^{-/-}$ mice. Representative H&E stained sections of colons from recipient mice as in Figure 1.(B) Flow cytometric analyses of Foxp3 expression in $CD4^+$ T cells recovered from indicated sites of WT and KO T cell recipients as in (A). Representative of analyses of 5 mice. (C) $CD4^+$ $CD62L^+$ $CD25^-$ $CD44^-$ naïve WT and *Bcl3*$^{-/-}$ T cells isolated via autoMACS were injected i.p. into *Rag1*$^{-/-}$ mice and recipients were weighed weekly and sacrificed 7 weeks post transfer when some mice were losing 20% of body weight (n=5 mice/group). (D) Overall appearance of the colons from WT and *Bcl3*$^{-/-}$ T cell recipients as in (C). (E) Representative flow cytometric analyses of indicated cytokine expression of $CD4^+$ T cells recovered from indicated sites of WT and *Bcl3*$^{-/-}$ T cell recipients as in (C). Sp, spleen; MLN, mesenteric lymph node; LP, colon lamina propria. Data represent means ± SD; * indicates $p < 0.05$.

Figure S2

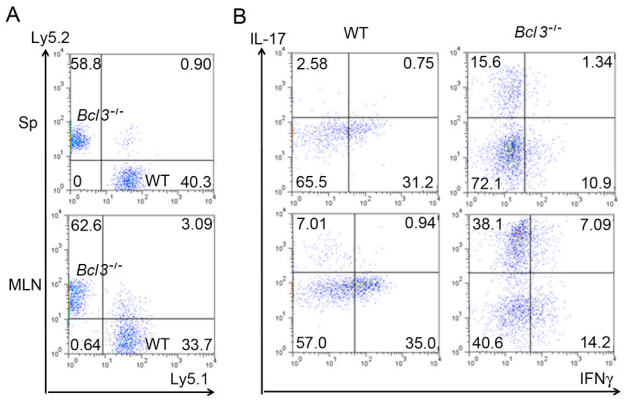

Figure S2. Bcl-3 functions cell-autonomously to control the T helper cell phenotype. Flow cytometric analyses of T cells recovered from *Rag1*$^{-/-}$ mice 4 weeks after co-transfer of WT Ly5.1 and *Bcl3*$^{-/-}$ Ly5.2 naïve CD4+ T cells, showing (A) relative amounts of *Bcl3*$^{-/-}$ and WT T cells recovered from sites shown and (B) expression of indicated cytokines in recovered *Bcl3*$^{-/-}$ and WT T cells. Representative of 5 independent co-transfer experiments.

Figure S3

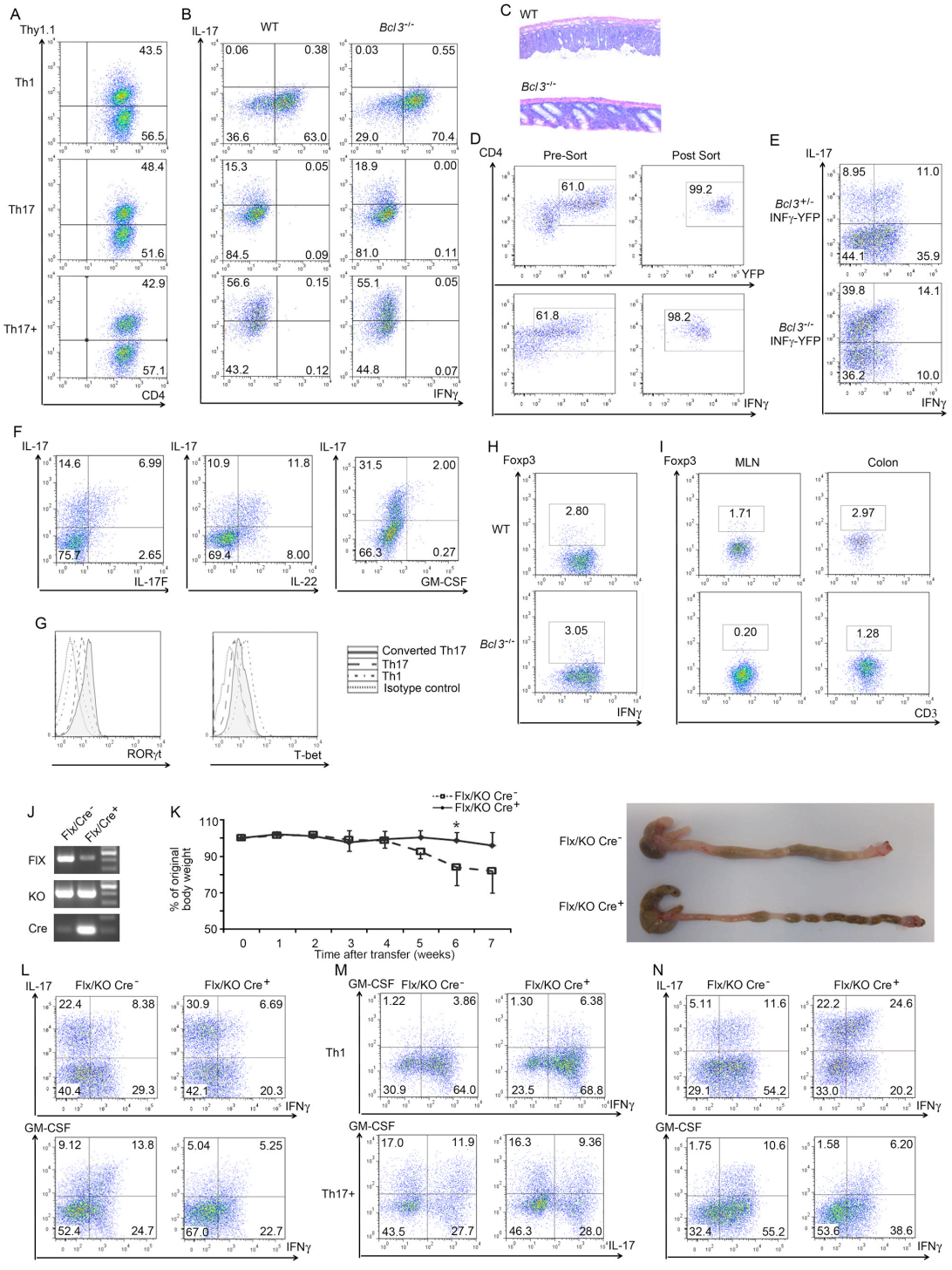

Figure S3. *Bcl3*$^{-/-}$ Th1 cells convert to non-pathogenic Th17 cells upon transfer. (A) Flow cytometric analyses of co-cultured WT Thy1.1 and *Bcl3*$^{-/-}$ Thy1.2 naive CD4^{+} T cells under Th1, Th17 or enhanced Th17 (Th17+) conditions *in vitro* and analyzed for (A) relative amounts of *Bcl3*$^{-/-}$ and WT T cells and (B) expression of indicated cytokines for *Bcl3*$^{-/-}$ and WT T cells. Representative of 5 independent experiments. (C)

H&E stained sections of colons from *Rag1*$^{-/-}$ mice recipients of WT or *Bcl3*$^{-/-}$ Th1 cells as in Figure 3B,C. (D) Flow cytometric analyses of pre- and post-sorted Th1 cells for indicated markers, with sorting based on expression of a IFNγ-YFP reporter; representative of heterozygous (Bcl-3$^{+/-}$) control (shown) and *Bcl3*$^{-/-}$ Th1 sortings. (E) Sorted YFP^{+} control and *Bcl3*$^{-/-}$ Th1 cells were transferred into *Rag1*$^{-/-}$ mice; shown are flow cytometric analyses of indicated cytokines in T cells from MLNs 4 weeks after transfer. Representative of 3 independent experiments. (F) Flow cytometric analyses of indicated cytokines in T cells recovered from MLNs of *Rag1*$^{-/-}$ mice 4 weeks after transfer of *Bcl3*$^{-/-}$ Th1 cells, representative of 3 independent experiments. (G) Flow cytometric analyses for RORγt and T-bet expression of T cells from (F) (*in vivo* converted Th17) and of *in vitro* generated WT Th1 and Th17 cells. (H) Flow cytometric analyses of WT and *Bcl3*$^{-/-}$ Th1 cells for indicated markers after exposure to Treg re-differentiation conditions *in vitro* for 3 weeks, representative of 3 independent experiments. (I) Representative flow cytometric analyses of 3 experiments for indicated makers of T cells recovered from indicated sites of *Rag1*$^{-/-}$ mice 4 weeks after transfer of WT and *Bcl3*$^{-/-}$ Th1 cells. (J) PCR analyses for the presence of loxP flanked (Flx) Bcl-3 in CD4^{+} T cells from Bcl-3$^{+/-}$ heterozygous controls [Flx/KO] and from mice conditionally ablated for Bcl-3 in T cells [Flx/KO; lck-Cre]. Right lane, size markers. (K) Naïve CD4^{+} CD45RB^{hi} CD25^{-} T cells isolated from control heterozygous [Flx/KO] mice and from mice conditionally ablated of Bcl-3 in T cells [Flx/KO; lck-Cre] were injected into *Rag1*$^{-/-}$ mice; shown are weight of recipient mice and appearance of colon at end of experiment (n=6 mice/group). (L) Representative flow cytometric analyses for indicated cytokines in CD4^{+} T cells recovered from colons of recipient mice in (K). (M) Representative flow cytometric analyses for indicated cytokines in T cells from control [Flx/KO] and T cell-ablated Bcl-3 mice [Flx/KO; lck-Cre] after *in vitro* differentiation under Th1 and Th17+ conditions. (N) Representative flow cytometric analyses for indicated cytokines in T cells recovered from MLN *Rag1*$^{-/-}$ mice 4 weeks after transfer of Th1 cells generated as in (M). Analyses shown in M and N are representative of 3 independent experiments.

Figure S4

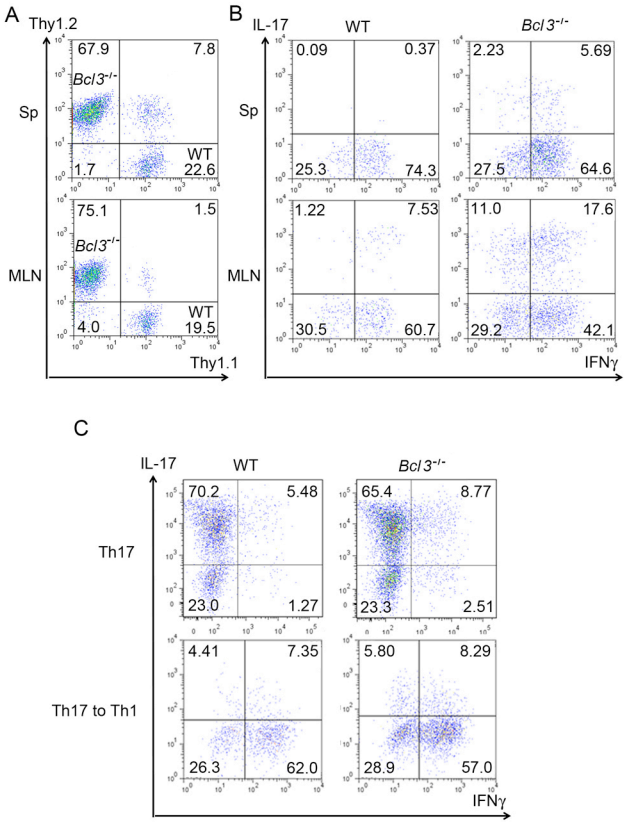

Figure S4. Cell-autonomous control of Th1 cell plasticity via Bcl-3. Flow cytometric analyses of T cells recovered from *Rag1*$^{-/-}$ mice 9 days after co-transfer of Thy1.1 WT and Thy1.2 *Bcl3*$^{-/-}$ Th1 cells showing (A) relative amounts of *Bcl3*$^{-/-}$ and WT T cells recovered from sites shown and (B) expression of indicated cytokines in *Bcl3*$^{-/-}$ and WT T cells. Representative of 3 independent experiments. (C) WT and *Bcl3*$^{-/-}$ Th17-differentiated cells (top panels) were subjected to two subsequent rounds of Th1 re-differentiation *in vitro* (bottom panels). Data shown representative of 3 independent experiments.

Figure S5

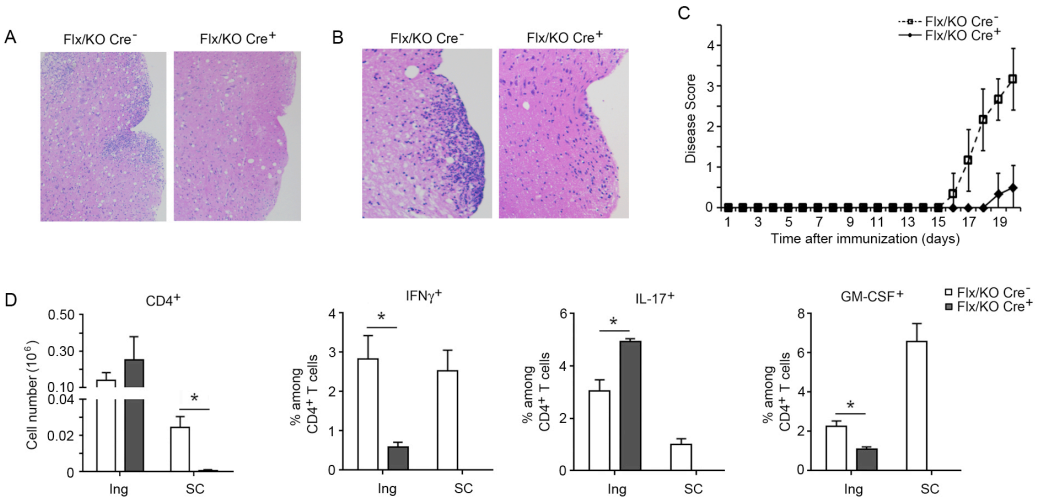

Figure S5. Bcl-3 controls Th1 cell plasticity and pathogenicity in experimental autoimmune encephalomyelitis. H&E staining of spinal cord sections of *Rag1*$^{-/-}$mice after adoptive transfer of T cells and a MOG peptide booster immunization as in Figure 5. Transferred T cells had previously been isolated from draining lymph nodes 10 days after immunization of heterozygous control mice [Flx/KO] and mice with T cell-specifically ablated Bcl-3 [Flx/KO; lck-Cre] and additional *in vitro* re-stimulation with MOG under Th1 conditions. (B) H&E staining of spinal cord sections of T cell-specific Bcl-3-deficient mice and control mice 20 days after initial MOG immunization (with booster immunization at day 7). (C) EAE disease scores of mice immunized and boosted as in (B) (n=6 mice/group). (D) Total numbers and cytokine production of CD4^{+} T cells recovered from inguinal lymph nodes (Ing) and spinal cords (SC) of mice at end of experiment as in (B) (n=6 mice/group). Data represent means ± SD; * indicates $p < 0.05$.

Figure S6

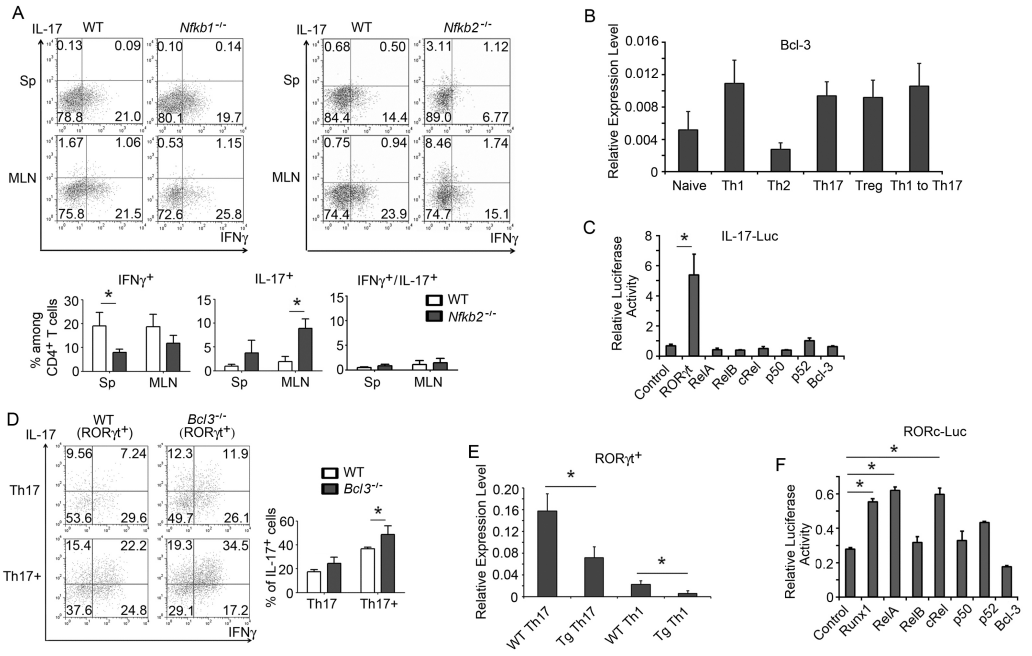

Figure S6. Mechanisms involved in Bc-3-mediated constraints of Th1 plasticity. (A) Representative flow cytometric analyses for indicated cytokine expression of T cells recovered from *Rag1*$^{-/-}$ mice from indicated sites 4 weeks after transfer of *in vitro* generated *NF-κB1*$^{-/-}$ KO and *NF-κB2*$^{-/-}$ Th1 cells. Summary data for transfer of *NF-κB2*$^{-/-}$ Th1 cells from 3 independent experiments shown below. (B) mRNA level of Bcl-3 in T cells after various *in vitro* T helper cell differentiation conditions as shown (n=3; similar results were obtained in an additional experiment with n=3). (C) IL-17-promoter-driven luciferase activity in Jurkat cells transfected with expression vectors for indicated NF-κB subunits and for the positive control RORγt and stimulated one day later with PMA plus ionomycin. Luciferase activity was measured four hours after treatment (n=3; similar results were obtained in an additional experiment with n=3). (D) Representative flow cytometric analysis of GFP^{+} T cells for indicated cytokine expression of *in vitro* generated WT and *Bcl3*$^{-/-}$ Th1 cells transduced with a GFP-RORγt retrovirus and subjected to re-differentiation under Th17 or Th17+ conditions for 1 week. Summary for IL-17-expressing cells from 3 independent experiments shown on the right. (E) *Rorc* mRNA level (RORγt) in WT and Bcl-3 transgenic (Tg) Th1 and Th17 cells (n=3; similar results were obtained in an additional experiment with n=3). (F) *Rorc* promoter-driven luciferase activity in Jurkat cells transfected with expression vectors for indicated NF-κB subunits and for the positive control Runx1 and stimulated one day later with PMA plus ionomycin. Luciferase activity was measured four hours after treatment (n=3; similar results were obtained in an additional experiment with n=3). Data represent means ± SD; * indicates $p < 0.05$.