SUPPLEMENTAL MATERIAL

Hofmann et al., http://www.jem.org/cgi/content/full/jem.20110128/DC1

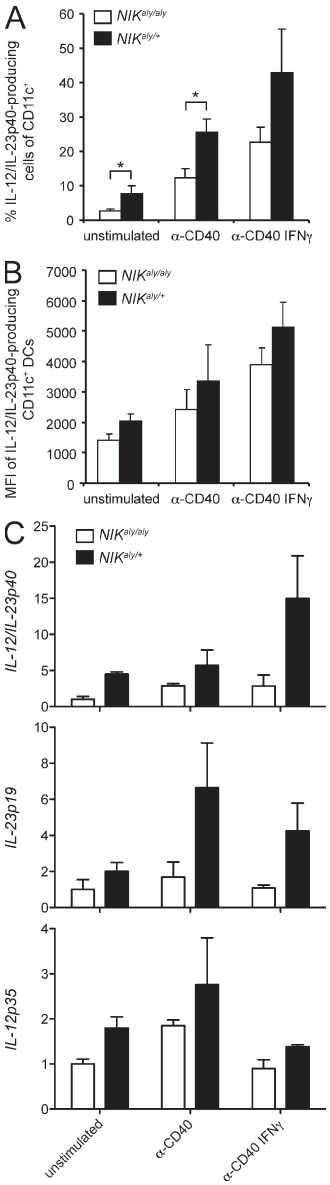

Figure S1. In vitro stimulated splenic DCs and their expression of IL-12/IL-23p40, IL-12p35, and IL-12p19. Splenic *NIK*$^{aly/aly}$ DCs were stimulated in vitro with α-CD40 and IFN-γ for 24 h. (A and B) GolgiPlug was added for the last 4 h of culture. Intracellular IL-12/IL-23p40 antibody FACS staining was performed together with cell surface staining for CD11c. The percentage of IL-12/IL-23p40–producing DCs (A) and the mean fluorescent intensities (MFI; B) are shown as means of at least two individual experiments. *, $P \leq 0.05$. (C) Splenic *NIK*$^{aly/aly}$ DCs were isolated and stimulated in vitro with α-CD40 and IFN-γ for 6 h. Cells were harvested, and whole RNA was isolated and reversely transcribed. qRT-PCR was performed to detect messenger RNA levels of IL-12/IL-23p40, IL-23p19, and IL-12p35. Shown are means of at least two independent experiments. (A–C) Error bars indicate SD.

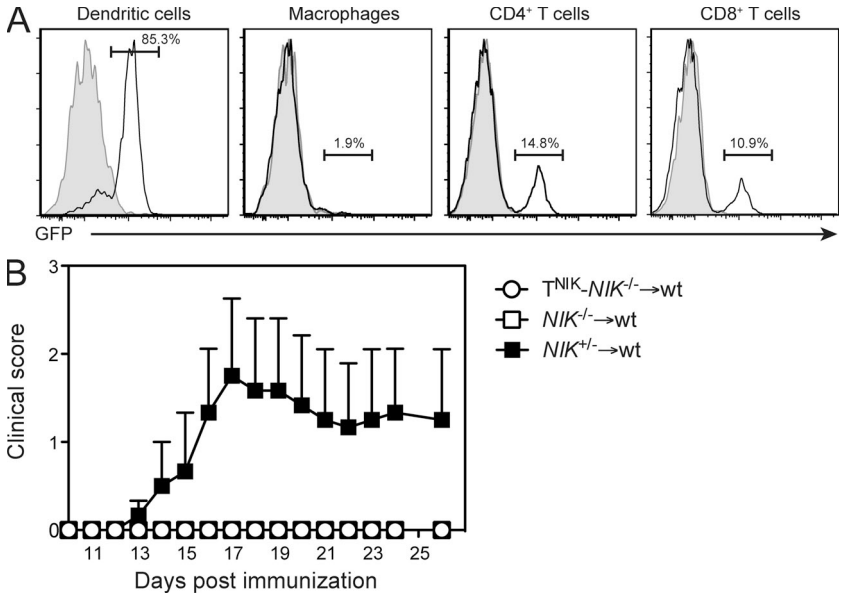

Figure S2. The transgenic GFP expression of DC^{NIK}-*NIK*$^{-/-}$ → WT BMCs and the EAE score of T^{NIK}-*NIK*$^{-/-}$ → WT BMCs. (A) Splenocytes of DC^{NIK}-*NIK*$^{-/-}$ → WT BMCs were analyzed by flow cytometry for the expression of GFP in CD11c$^+$ DCs, CD11b$^+$ macrophages, and CD8$^+$ and CD4$^+$ T cells. (B) T^{NIK}-*NIK*$^{-/-}$ → WT, *NIK*$^{+/-}$ → WT, and *NIK*$^{-/-}$ → WT BMCs were immunized with MOG_{35-55}/CFA and observed for clinical signs of EAE. Shown is one representative of two independent experiments ($n = 3$). Error bars indicate SEM.

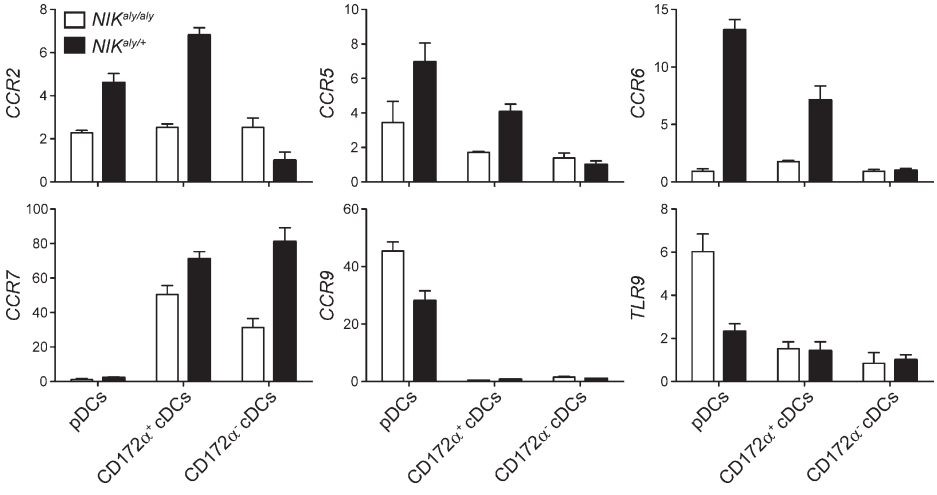

Figure S3. Expression of various chemokine receptors of thymic DC subsets. RNA of FACS-sorted thymic DC subsets from *NIK*$^{aly/aly}$ and *NIK*$^{aly/+}$ mice was transcribed into cDNA and analyzed by qRT-PCR for expression of different chemokine receptors and Toll-like receptors. Shown is fold change in expression level compared with CD172α$^-$ *NIK*$^{aly/+}$ cDCs, which was set to 1 for all genes except CCR7, in which fold change in expression level is compared with *NIK*$^{aly/aly}$ pDCs. Data are one representative of three independent experiments. Error bars indicate SD.

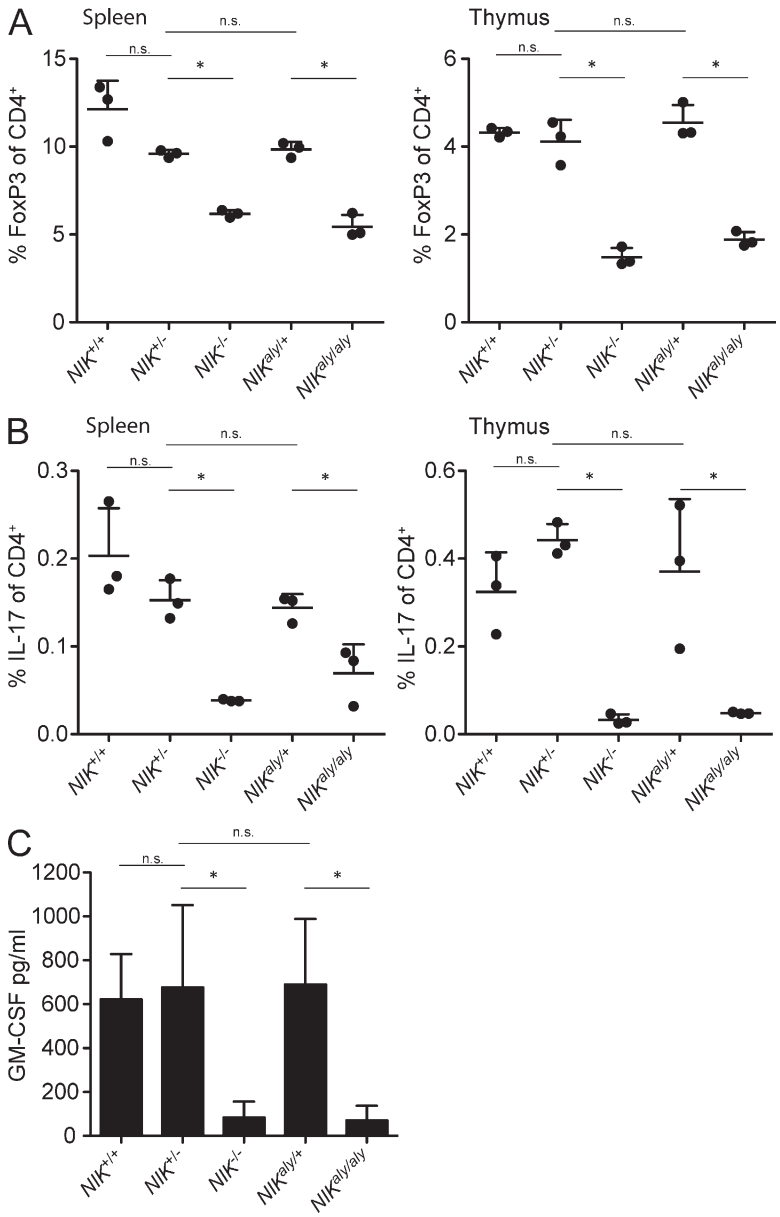

Figure S4. A phenotypic comparison of T cells from *NIK*$^{aly/aly}$, *NIK*$^{aly/+}$, *NIK*$^{-/-}$, *NIK*$^{+/-}$, and *NIK*$^{+/+}$ mice, in particular the expression of FoxP3 and effector cytokines. (A) CD4^{+}CD8^{-} cells in spleens (left) and thymi (right) of *NIK*$^{aly/aly}$, *NIK*$^{aly/+}$, *NIK*$^{-/-}$, *NIK*$^{+/-}$, and *NIK*$^{+/+}$ mice were analyzed by flow cytometry for the percentage of FoxP3-expressing T_{reg} cells ($n = 3$). (B) CD4^{+} splenic T cells (left) and CD4^{+} SP thymocytes (right) were stimulated in vitro with PMA and ionomycin and stained intracellularly for the secretion of IL-17 ($n = 3$). (C) CD4^{+} peripheral T cells of *NIK*$^{aly/aly}$, *NIK*$^{aly/+}$, *NIK*$^{-/-}$, *NIK*$^{+/-}$, and *NIK*$^{+/+}$ mice were stimulated in vitro with plate-bound α-CD3/α-CD28 for 48 h. GM-CSF secretion was analyzed by ELISA ($n = 3$). Data are one representative of two independent experiments. (A–C) Error bars indicate SD. *, $P \leq 0.05$.