Figure S1. **Generation of MARCH1<sup>fl/fl</sup> CD11c-CRE mice.** Shown are the maps of the MARCH1 exon6-targeting construct, loxP flanked allele, and CRE recombined/deleted allele. Red arrows indicate the primer pair used to determine the efficiency of Cre-mediated recombination.

Figure S2. **Surface expression of MHC II, B7 costimulatory molecules, and ICAM1 in thymic DCs.** (A) DCs were isolated from WT or MARCH1<sup>−/−</sup> mouse thymi and examined by flow cytometry. (B) DCs were isolated from WT or MHC II<sup>K>R/K>R</sup> mouse thymi and examined by flow cytometry. Data represent two independent experiments.

Figure S3. **Association of MHC II with the lipid raft in WT and MHC II<sup>K</sup>-deficient DCs.** BMDCs derived from WT or MHC II<sup>K>R/K>R</sup> mice were lysed with 1% Brij58 and fractionated by sucrose density gradient centrifugation as described in our previous study (Shin et al., 2000). Western blot analysis was performed by using antibodies raised against MHC II or actin. Note that some of MHC II molecules were fractionated into the light buoyant-density fractions (#4 and #5).
Figure S4. **Excessive accumulation of CD86 in DCs does not alter the lipid raft or tetraspanin web.** BMDCs derived from WT mice were transduced with retrovirus encoding CD86 (K>R) mutant along with cytosolic GFP. The surface level of CD86 in untransduced (GFP⁻) and transduced cells (GFP⁺) was determined by flow cytometry (A). Cells were stained using antibodies directed against indicated molecules or CTxB (for GM1) and subsequently analyzed by flow cytometry (B).
Figure S5. **TCR repertoire analysis and pathology of IBD mouse model.** (A) A table summarizing the TCR repertoire sequencing analysis. “>0.01% filtered TCRs” indicates that the TCR reads present >0.01% in at least one mouse of either genotype. Those TCRs were then further filtered to be characterized as “unique TCRs” if they were present in at least three of seven or eight mice in one genotype. (B) Principle component (PC) analysis of sequencing data. Treg cells and non-Treg CD4+ thymocytes are analyzed separately. Each dot represents each mouse. Note that dots are segregated under PC1 by genotype for both Treg cells and non-Treg CD4+ thymocytes. (C) Dot plot of unique non-Treg CD4+ thymocyte TCR frequencies in MARCH1−/− versus WT mice. Red and blue dots indicate unique TCRs significantly diminished and enriched in MARCH1−/− mice, respectively (P < 0.075, Mann-Whitney U test). ND, not detected. (D) The percentages of unique non-Treg CD4+ thymocyte TCR clones diminished or enriched significantly and more than fivefold in MARCH1−/− mice. (E) Mice described in Fig. 8 D were euthanized on day 56 after cell transfer, and the distal colons were subjected to histological examination. Representative microscopic images (200×) of hematoxylin and eosin-stained sections are shown.

Reference