Supplementary Data

Figure 1 Ndfip1 − / − mice develop gastrointestinal (GI) inflammation. (a) Gross anatomy of the small bowel of Ndfip1 − / − vs. Ndfip1 + / + littermate controls. (b) Histological analysis of the esophagus (Å~ 20), small bowel (Å~ 40), and colon (Å~ 40) of Ndfip1 − / − vs. Ndfip1 + / + littermate controls. (c) Weights of Ndfip1 − / − or Ndfip1 + / + littermates at different ages as indicated. Each point is the average weight of 3 – 5 different mice; * $P < 0.01$. (d) Flow cytometric analysis of cells from different sections of the GI tract. Cells from these organs were analyzed for their expression of CD4 + (T cells) and Siglec-F + (eosinophils). The data in a , b, and d are representative of > 5 independent experiments. (e) The percentages of eosinophils observed in d were compiled and analyzed. * $P < 0.05$, * * $P < 0.005$. Each dot represents an individual mouse. Mice were analyzed at 6 – 12 weeks of age.

Figure 2 T-cell activation and migration precedes eosinophilic infiltration into the gastrointestinal (GI) tract. (a) CD4 T cells from the spleen and lymph nodes of 4-week-old Ndfip1 − / − and Ndfip1 + / + littermates were analyzed for markers of activation (CD44 and CD62L). Activated T cells (CD44hi) are indicated by the gate and percentages of these cells are noted. (b) The percentages of eosinophils (Siglec-F +) and T cells (CD4 +) isolated from the esophagus of Ndfip1 − / − vs. Ndfip1 + / + littermate controls are shown. Data in a and b are representative of three different experiments. (c) The percentages of eosinophils in the esophagus of Nfip1 − / − and Ndfip1 + / + littermate control mice from all three experiments are graphed with each circle representing an individual mouse.

Figure 3 T cells are required for gastrointestinal (GI) inflammation in Ndfip1 − / − mice. (a) Weights of Ndfip1 + / + Rag1 − / − and Ndfip1 − / − Rag1 − / − mice are shown. Each data point includes weights from 3 to 5 different mice. (b) Histological analysis of the esophagus (Å~ 20), small bowel (Å~ 40), and colon (Å~ 40) from Rag1 − / − Ndfip1 − / − vs. Ndfip1 − / − animals. Data shown are representative of at least three mice of each genotype.

Figure 4 T cells in Ndfip1 − / − mice are more likely to make interleukin (IL-5). (a) Serum levels of IL-5 measured by enzyme-linked immunosorbent assay (ELISA). Each circle represents an individual mouse. The data include 12 Ndfip1 − / − , 12 Ndfip1 + / + littermates, and 3 Ndfip1 − / − RAG − / − mice. (b) IL-5 from spleen or lymph node cells isolated from Ndfip1 − / − (black) or Ndfip1 + / + (white) littermates. The graph shows the average and s.d. from 3 to 4 different experiments. (c) Intracellular stain of IL-5 in gated CD4 T cells isolated from the spleen of Ndfip1 − / − or Ndfip1 + / + littermates. * $P < 0.05$, * * $P < 0.0005$.

Figure 5 Ndfip1 − / − CD4 T cells are sufficient to induce gastrointestinal (GI) inflammation. Naive CD4 T cells were sorted from spleen and lymph nodes of Ndfip1 − / − or Ndfip1 + / + littermates and transferred to Rag1 − / − mice. (a) The percent weight change in mice that received either naïve Ndfip1 − / − (black) or Ndfip1 + / + (white) CD4 T cells is shown. Each point in the graph represents the weight of an individual mouse as a percentage of its initial weight at the time of transfer. (b) Histological analysis of GI sections from mice that received naive Ndfip1 − / − or Ndfip1 + / + CD4 + T cells. (c) Serum interleukin-5 (IL-5) levels were analyzed by enzyme-linked immunosorbent assay (ELISA) at 5 to 6 weeks after transfer of Ndfip1 − / − or Ndfip1 + / + CD4 T cells. Each point in the graph represents one mouse. (d) Levels of IL-5 produced by anti-CD3-stimulated spleen and lymph node cultures from mice that received Ndfip1 − / − (black) or Ndfip1 + / + (white) cells. *$P < 0.05$.

Figure 6 Itch mutant mice have a less severe gastrointestinal (GI) phenotype. (a) Histological analysis of the GI tract from Itch-deficient mice and wild-type (WT) mice at 4 to 5 months of age. (b) The percentage of eosinophils determined by flow cytometric analysis of cells isolated from different GI sections from 4 to 5 months old Itch-deficient mice and age-matched controls. (c) Serum levels of interleukin-5 (IL-5) from Ndfip1 − / − , WT, or Itch-deficient mice at different ages measured by enzyme-linked immunosorbent assay (ELISA). Each point in the graph represents an individual mouse. The data include 12 Ndfip1 − / − , 3 Itch-deficient mice, and 3 WT mice for each age indicated. (d) Levels of IL-5 produced from anti-CD3 stimulated spleen cultures measured by ELISA. Intracellular cytokine staining for IL-4 and IL-5 in T helper type 2 (T H 2)-differentiated CD4 T cells from WT, Itch mutant, or Ndfip1 − / − mice. Activation status of gated spleen CD4 + T cells from mice that are WT, Itch mutant, or Ndfip1 − / − at 5 – 7 weeks of age. Gate represents CD44 high cells. Same as c but including five mice of each genotype. * $P < 0.01$.

Figure 7 Single-nucleotide polymorphism (SNP) analysis. In all, 37 different SNPs that span the region including 50 kb before the start site of Nedd4 family interacting protein 1(Ndfip1) and 50 kb downstream of the coding region were analyzed. (a) Each SNP is represented by a dot at the indicated locations on human chromosome 5. Five of the analyzed SNPs showed significant differences between a population of healthy individuals and inflammatory bowel disease (IBD) patients and are represented by arrows, which show their location with respect to the Ndfip1 gene. (b) A linkage disequilibrium plot of all 37 SNPs was analyzed. The locations of the SNPs analyzed are indicated. Each square shows the linkage disequilibrium between two SNPs. Darker squares indicate a higher linkage disequilibrium.