Supplemental Data

Ndfip1 Protein Promotes the Function of Itch Ubiquitin Ligase to Prevent T Cell Activation and T Helper 2 Cell-Mediated Inflammation

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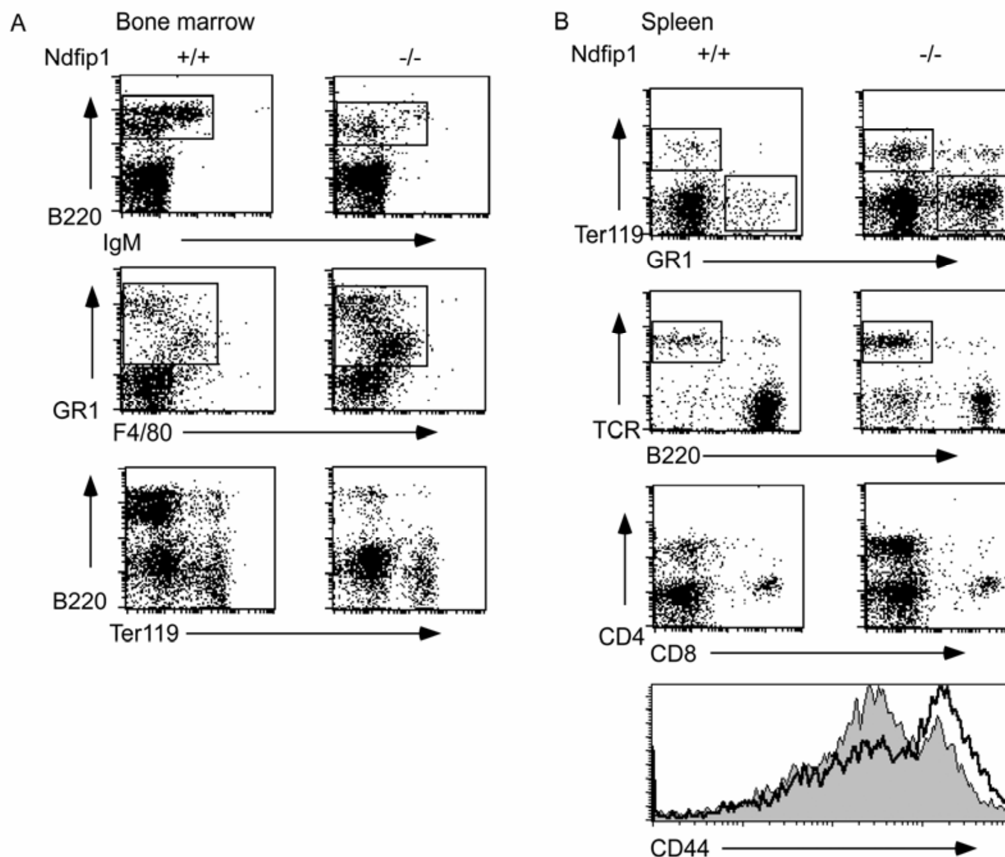

Figure S1. Mice Lacking Ndfip1 Exhibit Multiple Defects in Cells of the Hematopoietic System
Bone marrow (A) and spleen cells (B) from 12 week old *Ndfip1*$^{-/-}$ and *Ndfip1*$^{+/+}$ were stained and analyzed by flow cytometry. Histogram shows TCR^{+} cells from the spleen. The thick line represents *Ndfip1*$^{-/-}$ cells while the thin line shows *Ndfip1*$^{+/+}$ cells. FACS plots are representative data from one of four mice of each genotype that were analyzed in this manner.

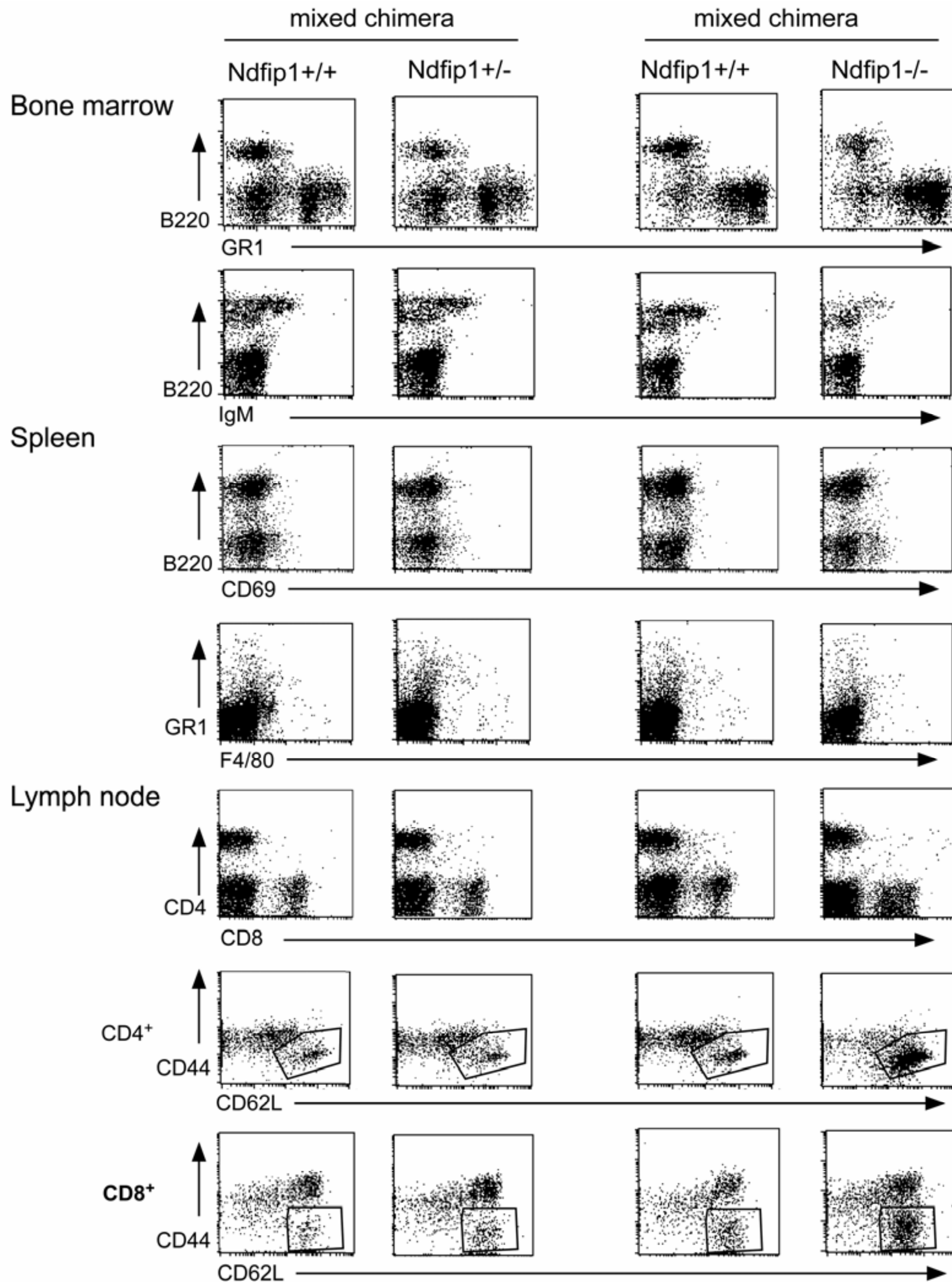

Figure S2. *Ndfip1*$^{+/-}$ Cells Are Phenotypically Similar to *Ndfip1*$^{+/+}$ Cells
Mixed bone marrow chimeras were made using an equal mixture of *Ndfip1*$^{+/+}$ (Ubi-GFP Tg) cells and either *Ndfip1*$^{+/-}$ or *Ndfip1*$^{-/-}$ cells. 5-6 weeks after reconstitution, cells from various organs were isolated and analyzed by flow cytometry. Adjacent vertical columns represent cells coming from the same mixed chimera. Boxed regions highlight activated T cells. These data are representative of four mice from each set of mixed chimeras.

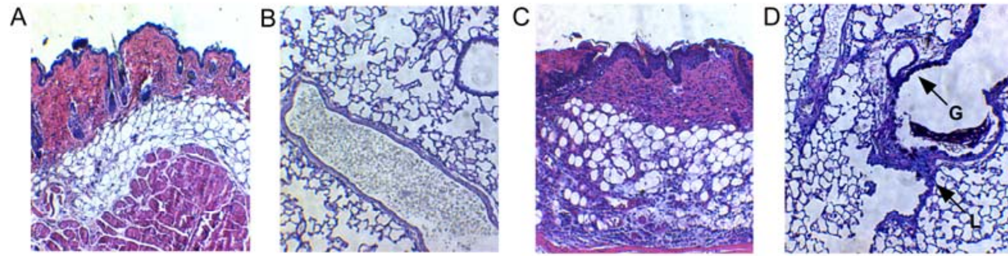

Figure S3. Immunization with OVA + Alum Exacerbates Inflammatory Disease

Mice were reconstituted with *Ndfip1*$^{+/+}$ or *Ndfip1*$^{-/-}$ bone marrow and 5 weeks later were immunized with OVA + Alum. 8 days after immunization, mice were sacrificed and sections were analyzed for inflammation. *Ndfip1*$^{+/+}$ (A) skin and (B) lung and *Ndfip1*$^{-/-}$ (C) skin and (D) lung sections were stained with H and E (skin) and PAS (lung) to demonstrate the extent of inflammation within these organs. G=goblet cells and L=lymphocytic cells.

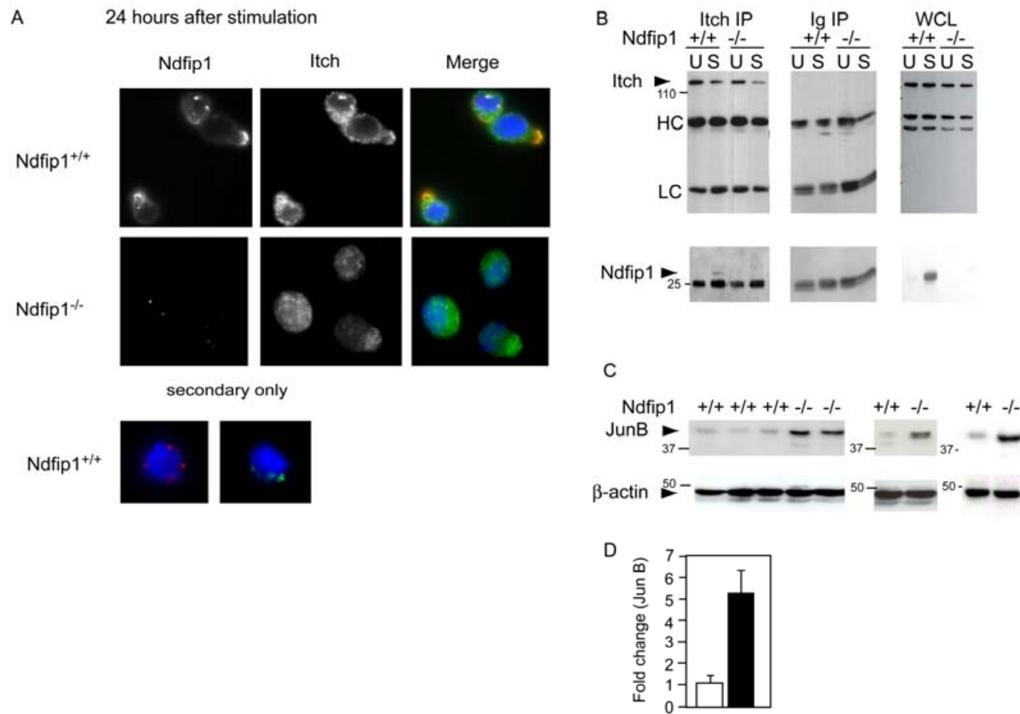

Figure S4. Immunofluorescence and IP Controls
(A) Wild-type T cells were stimulated for 24 hours with anti-CD3 and anti-CD28 and then fixed, permeabilized and stained for Ndfip1 (+ anti-hamster CY5) and Itch (+ anti-mouse AF488) or with secondary antibodies alone. (B) *Ndfip1*$^{+/+}$ and *Ndfip1*$^{-/-}$ T cells were cultured in media alone (U) or stimulated 24 hours with anti-CD3 and anti-CD28 (S). Cells were then lysed and immunoprecipitated with anti-Itch (Itch IP) or an IgG1 isotype control (Ig IP). Blots were probed with anti-Itch or anti-Ndfip1. (C) Whole cell lysates of CD4$^+$ T cells isolated from *Ndfip1*$^{+/+}$ or *Ndfip1*$^{-/-}$ mice were analyzed by immunoblot for JunB or β-actin (loading control). (D) To determine the fold change of JunB, amounts of JunB protein were normalized to β-actin and the ratio was then compared between the two genotypes. White bar represents *Ndfip1*$^{+/+}$ and black bar represents *Ndfip1*$^{-/-}$. Error bars represent standard errors. LC=light chain, HC=heavy chain of the immunoprecipitating antibody.

F2 genotyping results				
	+/+	+/-	-/-	Total
Male	41	67	18	126
Female	40	75	29	144
Total	81	142	47	270
percent	30%	53%	17%	
Expected	67.5	135	67.5	

0.00961 chi square test
0.9952 chi distribution