Supporting Information

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SI Materials and Methods

Sequence of Primers Used for Amplifying *Ndfip1* cDNA.

Primer	Sequence (5′→3′)
Ndfip1 sense	TTGAATTCGCCACCATGGCCTTGGCGTTGGCG GCGCTG
Ndfip1 antisense	AAAACTCGAGTTAATAAATAAAGAGAACTC TGG

Ndfip1, neural precursor cell expressed, developmentally down-regulated protein 4 family-interacting protein 1.

Sequence of the Primer Used for Sequencing Subcloned Ndfip1 cDNAs in pMX-IRES-GFP Vector.

Primer	Sequence (5′→3′)
pMX-SS1	GACGGCATCGCAGCTTGGATACAC

IgE ELISA. Plates were coated overnight with 1 μg/mL anti-IgE capture antibody (clone R35-72; BD). The capture antibody was removed, plates were blocked with 1% BSA, and washed, and then plasma samples (diluted 1:20) and standards were added, followed by a 1-h incubation at 37 °C. Plates were then washed, and 2 μg/mL biotinylated anti-IgE detection antibody (clone R35-118; BD) was added. Binding was detected by subsequent incubation with streptavidin-conjugated alkaline phosphatase (Vector Laboratories), followed by the addition of phosphatase substrate tablets (Sigma) in phosphatase/glycine buffer. Plates were developed for 1 h at 37 °C, and optical densities at 405 nm were measured using a plate reader (Molecular Devices). IgE quantification was calculated according to calibration curves based on the optical densities for a series of dilutions of a control IgE isotype antibody of known concentration (clone C38-2; BD).

Flow Cytometry. For carboxyfluorescein diacetate succinimidyl ester (CFSE), cells were suspended at 10^6 per milliliter in PBS containing 10% FCS. An equal volume of 2 μM CFSE in PBS solution was then added with mixing (final concentration, 1 μM), and cells were incubated in a 37 °C water bath for 5–10 min and washed with cold complete RPMI (cRPMI) before use.

For cell trace violet (CTV) labeling before adoptive transfer of T cells, splenocytes suspended at 10^8 cells per milliliter in RPMI containing HI-FCS (10% vol/vol) were transferred to the base of a fresh 15mL conical tube; 1 μL of CTV stock solution (10 mM) per milliliter of cell suspension was placed on the dry wall of the tubes, and then tubes were capped, inverted, and briefly vortexed (final CTV concentration, 10 μM). After 5 min of incubation in the dark, 10 mL of 10% FCS/RPMI was added, and then cells were sedimented by centrifugation before another wash in 10 mL of the same medium. Cells were then resuspended in PBS and passed through a 70-μm cell strainer (BD) before i.v. injection (200 μL per mouse).

Lymphocytes from spleen were plated at 5×10^5 to 4×10^6 (a fixed value within this range for any given experiment). For cytokine staining, cells were plated at 10^5 per well in 96-well tissue culture plates in 200 μL of cRPMI containing phorbol myristate acetate (PMA) (50 ng/mL; Sigma), ionomycin (500 ng/mL; Sigma), and GolgiStop (1/1,000; BD) and left to accumulate cytokines for 3–4 h at 37 °C. Cells were surface-stained for 20 min at 4 °C with antibodies diluted in PBS containing 0.5% FCS (Gibco) and 2 mM EDTA. Where required, cells were then washed and resuspended in a secondary antibody for 20 min at 4 °C. For intracellular staining, cells were then fixed and permeabilized using the Fixation/Permeabilization kit (eBioscience) and stained for 20 min with intracellular antibodies and, where necessary, BrilliantViolet605-Streptavidin (BioLegend). After staining, cells were washed twice and read on an LSR II flow cytometer (BD). Flowjo software (Treestar) was used for analysis.

Antibodies Used for Surface Staining.

Antigen	Clone	Conjugate	Source
B220	RA3-6B2	PE-Cy7	BD
B220	RA3-6B2	APC-Cy7	BioLegend
CD4	RM4-5	Alexa Fluor 700	BioLegend
	GK1.5	APC-Cy7	BD
	GK1.5	Biotin	BD
CD8	53-6.7	APC-Cy7	BioLegend
CD44	IM7	Pacific blue	BioLegend
CD45.1	A20	PE	BD
CD45.1	A20	Alexa Fluor 700	BioLegend
CD45.2	104	PerCP-Cy5.5	BD
GITR	DTA-1	Biotin	BioLegend
CD25	PC61.5	PE	eBioscience
	PC61	APC	BD
TCRβ	H57-597	Biotin	BD
CD11b	M1/70	Biotin	BD
CD86	GL1	PE	BD Pharmingen
CD23	B3B4	PE-Cy7	eBioscience

Antibodies Used for Intracellular Staining.

Antigen	Clone	Conjugate	Source
Foxp3	FJK-16s	A700, FITC or eFluor 450	eBioscience
GATA-3	L50-823	Alexa Fluor 488	BD
IFN-γ	XMG1.2	APC	eBioscience
IL-17A	TC11-18H10.1	FITC	BioLegend
IL-4	BVD6-24G2	PE-Cy7	eBioscience
JunB	C37F9	none	Cell Signaling
Ki-67	B56	Alexa Fluor 647	BD

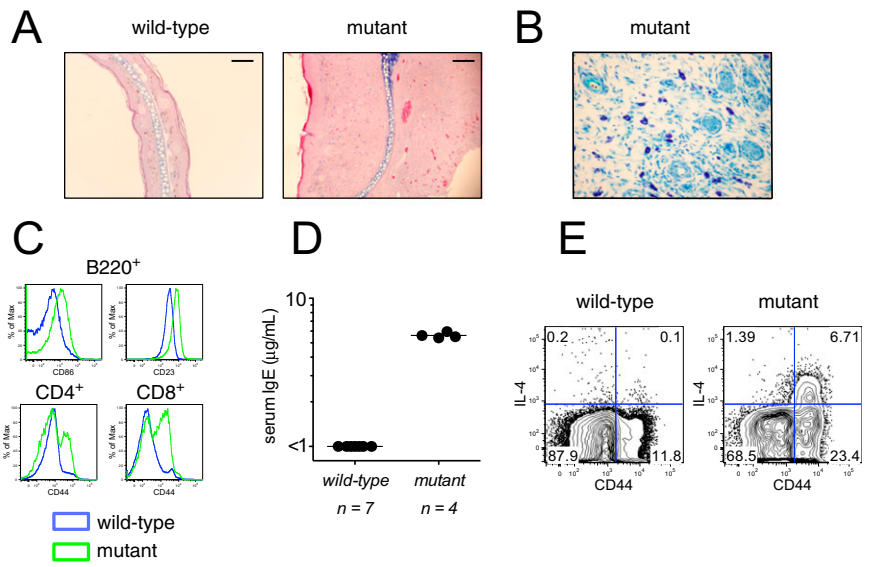

Fig. S1. (*A*) Representative H&E-stained sections of skin from wild-type (*Left*) and mutant (*Right*) mice. (*B*) Representative Alcian blue-stained section from skin of a mutant mouse, showing infiltration of mast cells. (*C*) Representative flow cytometric staining of lymph node cells from mutant (green) and wild-type (blue) mice, showing cell surface CD86 and CD23 on B cells and CD44 on $CD4^+$ or $CD8^+$ T cells. (*D*) Serum IgE concentrations in individual mutant or wild-type mice. (*E*) Representative flow cytometric staining for CD44 and intracellular IL-4 on gated $CD4^+$ lymph node cells from mutant or wild-type mice, stimulated ex vivo with PMA for 3 h.

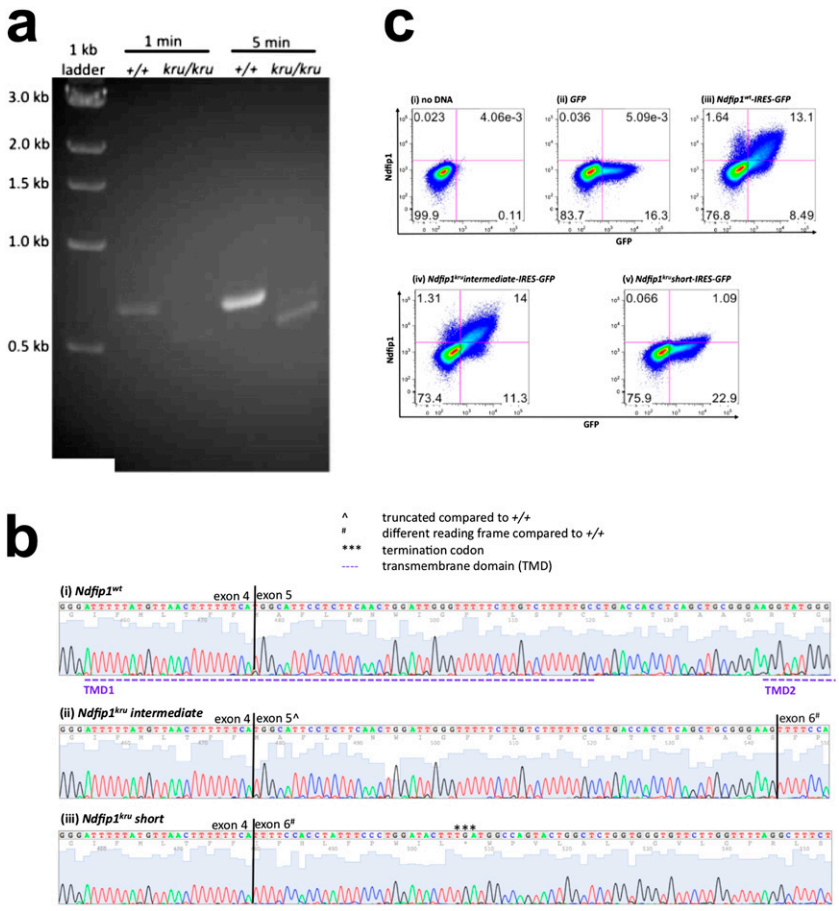

Fig. S2. Effect of Ndfip1^{kru} splice site mutation on mRNA and protein. (*A*) Agarose gel electrophoresis showing products obtained by PCR amplification of cDNA from wild-type and *Ndfip1*$^{kru/kru}$ splenocytes using primers flanking the start and stop codons of *Ndfip1*. (*B*) Capillary traces showing the relevant sequence region of the cDNAs amplified in *A*. Vertical bars denote the position of splice sites, and amino acids in green denote sequence that is not present in wild-type Ndfip1. (*C*) Flow cytometric staining for Ndfip1, detected by a monoclonal antibody raised to a conserved Ndfip1 N-terminal peptide, versus GFP expression in HEK293 cells transduced with bicistronic GFP vectors containing cDNAs encoding the indicated Ndfip1 variants.

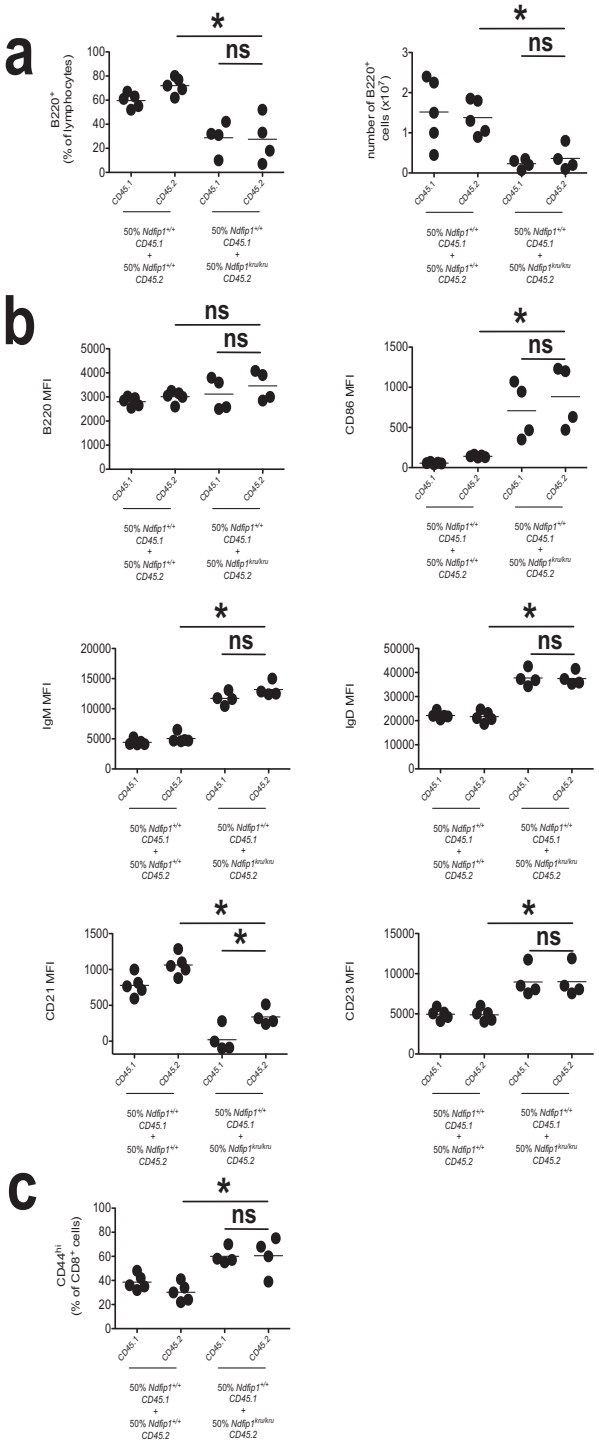

Fig. S3. Effects of Ndfip1 deficiency on the phenotype of B cells and CD8 T cells. Mixed bone marrow chimeras were constructed as in Fig. 2 by transplantation of irradiated *Rag1*$^{-/-}$ mice with a 50:50 mixture of *Ndfip1*$^{kru/kru}$ *CD45.2* bone marrow and *Ndfip1*$^{+/+}$ *CD45.1* bone marrow (50% kru) or a control 50:50 mixture of *Ndfip1*$^{+/+}$*CD45.2* bone marrow and *Ndfip1*$^{+/+}$ *CD45.1* bone marrow (100% wt). (*A*) Frequency and absolute number of CD45.1^{+} and CD45.2^{+} B cells in the spleens of individual chimeras. (*B*) Expression of the markers B220, CD86, IgM, IgD, CD21, and CD23 on CD45.1^{+} and CD45.2^{+} B cells. MFI, geometric mean fluorescence intensity. (*C*) Percentage of CD44^{hi} effector/memory cells among CD45.1^{+} and CD45.2^{+} CD8^{+} T cells. *$P < 0.05$ by ANOVA, followed by Bonferroni posttest. ns, not significant.

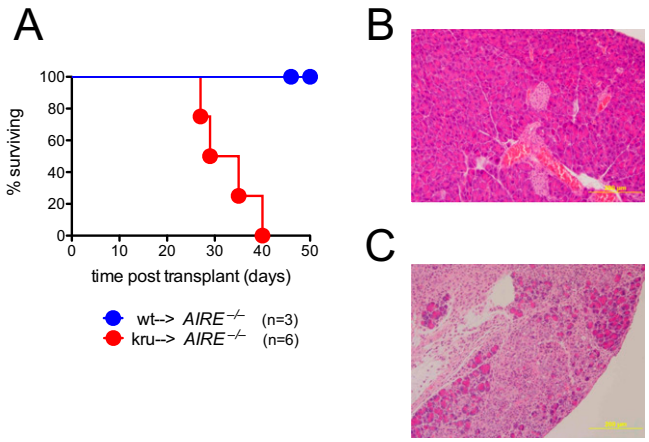

Fig. S4. Ndfip1 deficiency cooperates with Aire deficiency to precipitate autoimmune pancreatitis. B10.BR *Aire*$^{-/-}$ mice were irradiated and transplanted with either *Ndfip1*$^{kru/kru}$ or wild-type B10.BR bone marrow. (*A*) Survival of recipients posttransplantation. (*B* and *C*) Representative histology of the pancreas in recipients of wild-type (*B*) or *Ndfip1*$^{kru/kru}$ (*C*) bone marrow.