SUPPLEMENTAL MATERIAL

Kamizono et al., http://www.jem.org/cgi/content/full/jem.20092176/DC1

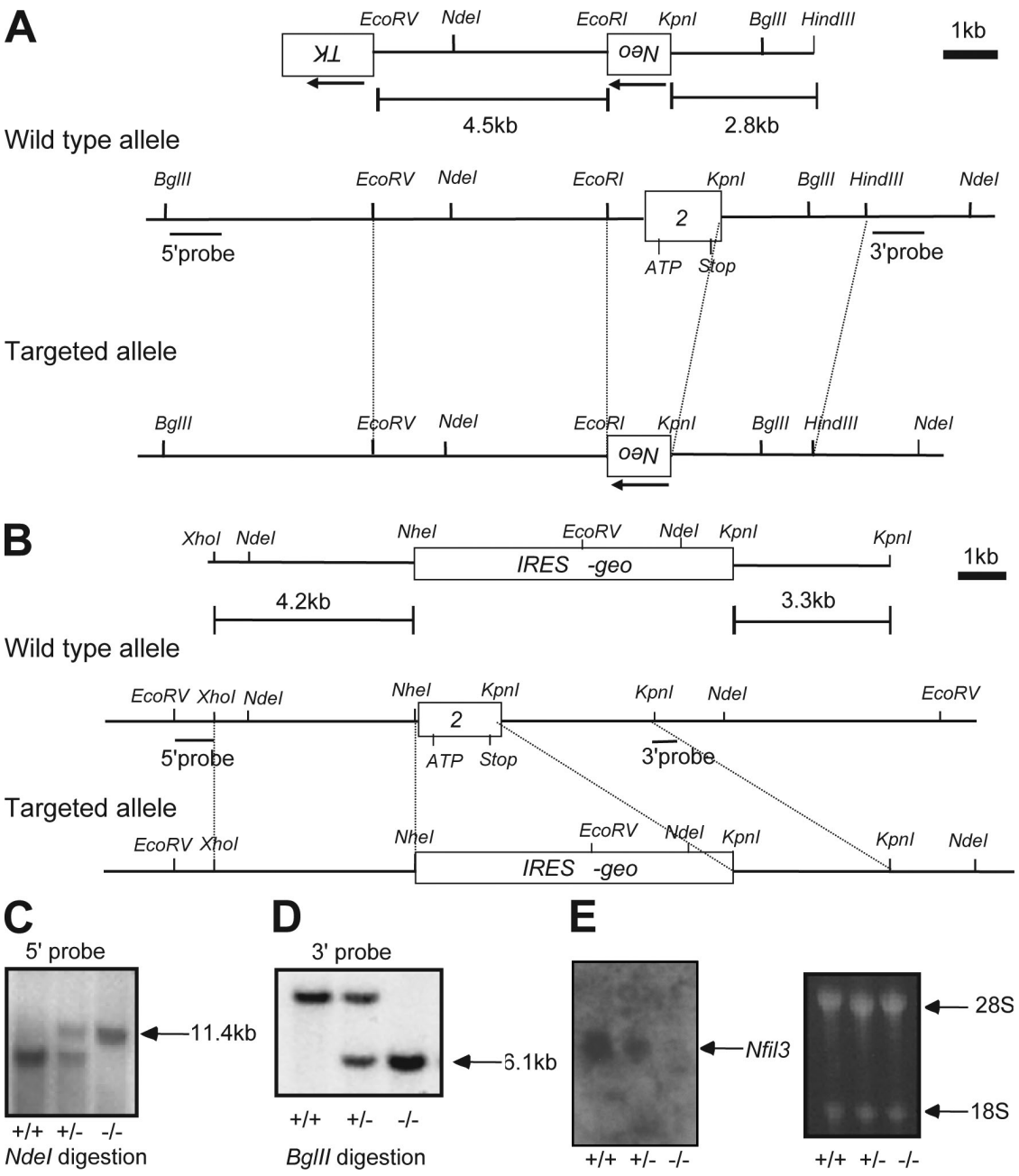

Figure S1. Generation of *Nfil3*$^{-/-}$ and *Nfil3*$^{+/-}$*-IRES β-geo* mice. (A) *Nfil3*$^{-/-}$ mice. The WT murine *Nfil3* locus, the *neo* cassette targeting vector, and the targeted *Nfil3* allele in which *Nfil3* exon 2 was replaced with the *neo*^{R} cassette are shown. The 5′ and 3′ probes used in Southern blotting to identify homologous recombinants are indicated. (B) *Nfil3*$^{+/-}$*-IRESβ-geo* mice. The WT murine *Nfil3* locus, the *IRESβ-geo* targeting vector, and the targeted *Nfil3 IRESβ-geo* allele in which *Nfil3* exon 2 was replaced with *IRESβ-geo* are shown. The 5′ and 3′ probes used in Southern blotting to identify homologous recombinants are indicated. (C and D) Southern blot analysis of tail DNA of F1 progeny of *Nfil3*$^{+/-}$ intercrosses. Identification of *Nfil3*$^{+/+}$, $^{+/-}$, and $^{-/-}$ progeny using either the 5′ (C) or 3′ (D) probes indicated in A is shown. (E) Northern blot analysis of RNA from the progeny in C and D at E8.

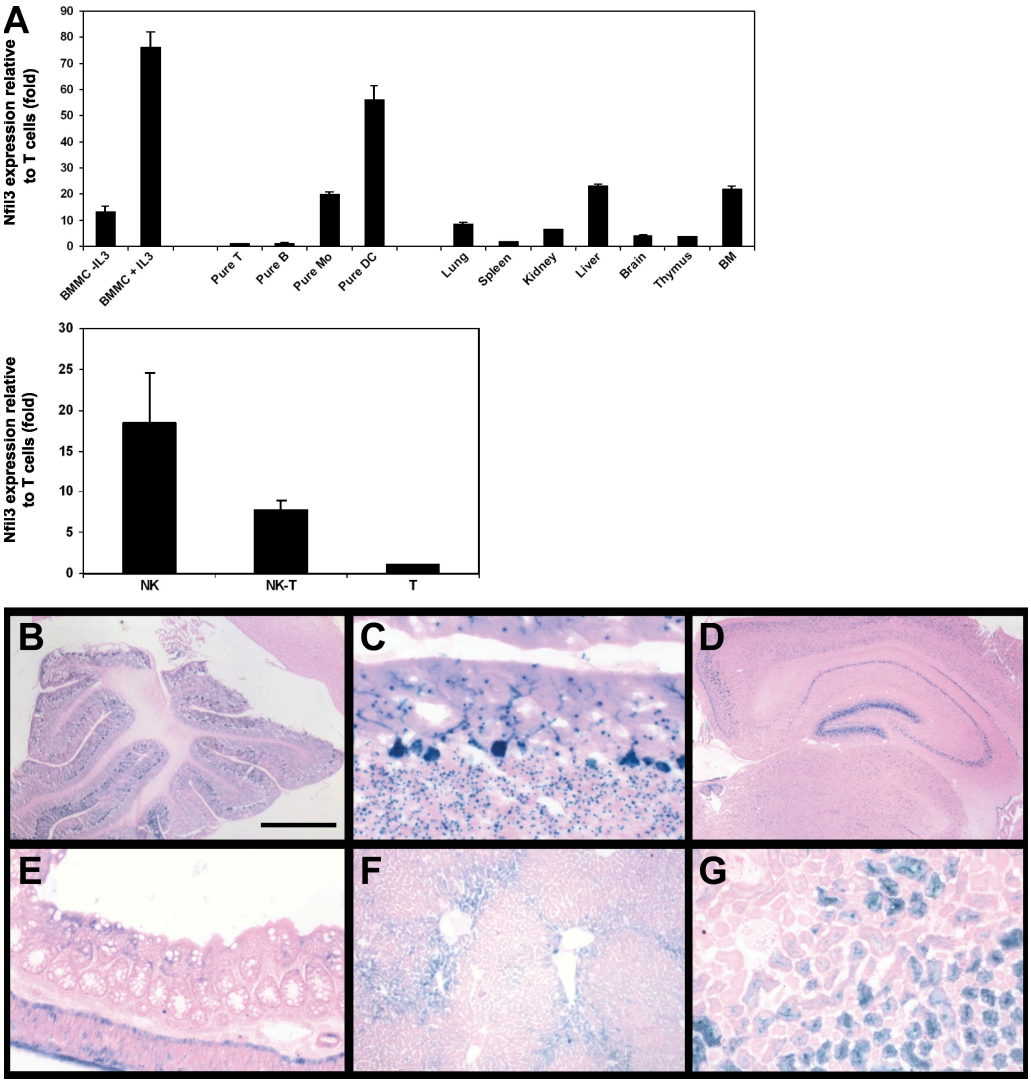

Figure S2. Endogenous *Nfil3* expression patterns. (A) Real-time RT-PCR analysis of *Nfil3* mRNA expression in purified cell populations and mouse tissues (top) and sorted BM NK, NK T, and $CD3^+$ T cells (bottom). Levels of mRNA are expressed relative to those in T cells. Data shown are the mean ± SD for triplicate determinations. (B–G) X-Gal staining to detect Nfil3 protein expression in tissues of *Nfil3*$^{+/-}$*-IRES*β*-geo* mice. In the brain, prominent X-Gal staining is seen in the complete cell bodies and dendrites of Purkinje cells in the cerebellum (B and C) and in the olfactory bulbs, hippocampus, and dentate and cingular gyri (D). Strong X-Gal staining is also present in the longitudinal and circular muscle layers in the bowel wall (E), the peripheral lobular cells of the liver (F), and the medulla of the kidney (G). Bars: (B, D, E, and G) 1,000 µm; (C) 100 µm; (F) 500 µm.

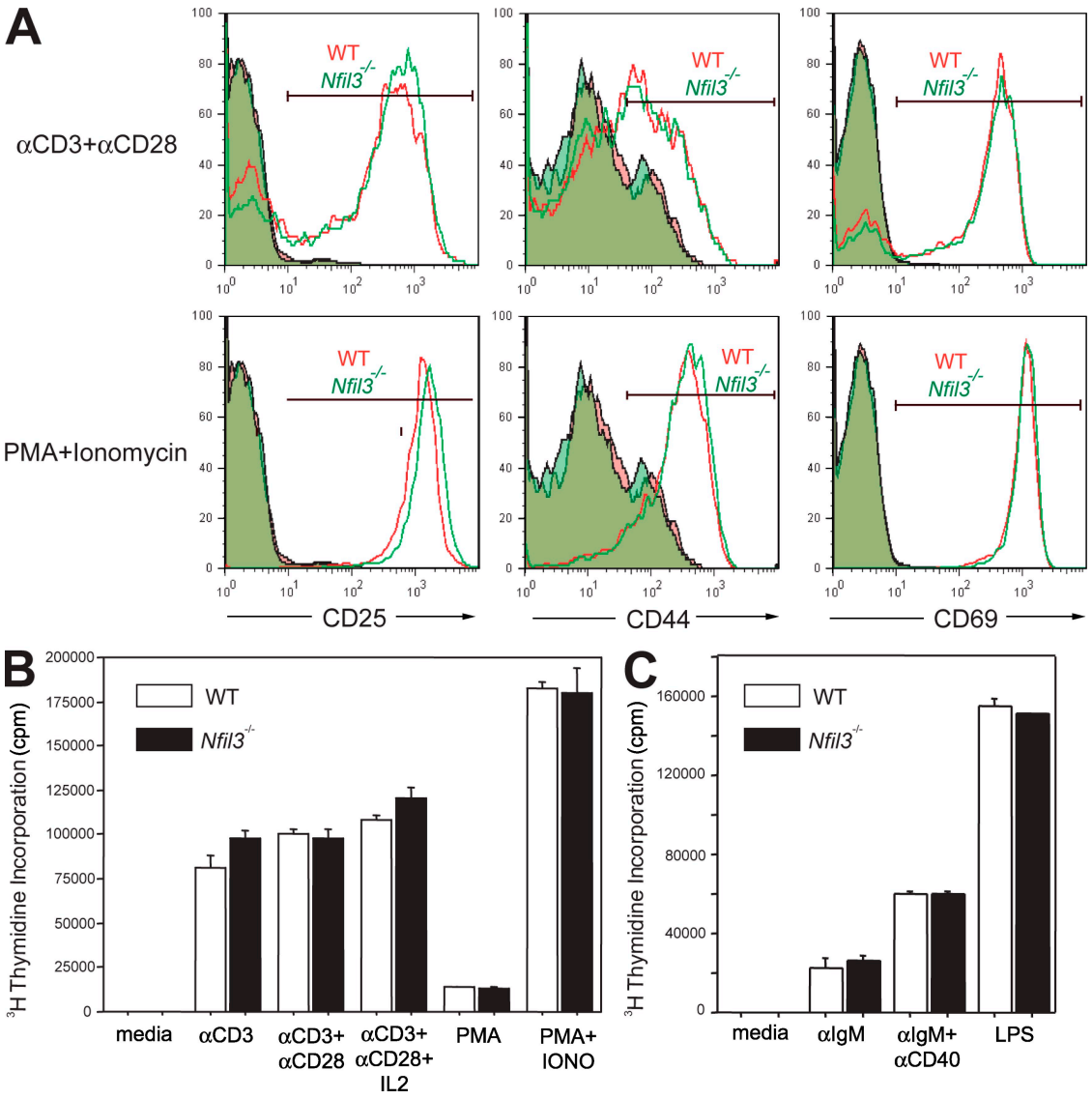

Figure S3. Functional analysis of splenic T cells and thymocytes in *Nfil3*$^{-/-}$ mice. Normal T cell activation. Splenic T cells from WT and *Nfil3*$^{-/-}$ mice were examined for the expression of the indicated T cell activation markers by flow cytometry (n = 3 mice per group). Data shown are representative of three independent experiments. (B) Normal T cell proliferation. WT and *Nfil3*$^{-/-}$ splenic T cells were stimulated as indicated and proliferation was determined by ^{3}H-thymidine incorporation. No significant differences in the activation or proliferation of WT and *Nfil3*$^{-/-}$ mature T cells were observed. (C) Normal B cell proliferation. WT and *Nfil3*$^{-/-}$ splenic B cells were stimulated as indicated and proliferation was determined by ^{3}H-thymidine incorporation. No significant differences in the activation or proliferation of WT and *Nfil3*$^{-/-}$ mature B cells were observed. For B and C, data shown are the mean ^{3}H-thymidine incorporation ± SD for triplicate determinations (n = 3 mice per group) and are representative of three independent experiments.

Table S1. Comparison of numbers of NK and NK T cells in various organs of WT and *Nfil3*$^{-/-}$ mice

Marker and organ	WT	*Nfil3*$^{-/-}$
NK1.1^{+}CD3^{-}		
Spleen	$19.63 \pm 5.41 \times 10^5$	$0.56 \pm 0.05 \times 10^{5**}$
Liver	$2.15 \pm 0.76 \times 10^5$	$0.12 \pm 0.04 \times 10^{5**}$
Lung	$4.09 \pm 1.23 \times 10^5$	$0.23 \pm 0.14 \times 10^{5**}$
NK1.1^{+}CD3^{+}		
Spleen	$12.50 \pm 3.28 \times 10^5$	$11.53 \pm 1.82 \times 10^5$
Liver	$8.44 \pm 1.54 \times 10^5$	$11.92 \pm 6.66 \times 10^5$
Lung	$0.86 \pm 0.63 \times 10^5$	$1.28 \pm 0.55 \times 10^5$

WT and *Nfil3*$^{-/-}$ mice were examined at about 6 wk of age. Data shown are the mean absolute number of cells ± SD of three independent determinations. *, $P < 0.05$; **, $P < 0.01$ (unpaired Student's *t* test).