Supporting Information

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SI Materials and Methods

Generation of Transgenic Mice. Human TLR3 cDNA was cloned into pCAGGS at the XhoI site (1). The DNA fragment containing TLR3 under the control of the β-actin promoter released by digestion with SalI and HindIII was gel-purified and microinjected into the pronucleus of fertilized eggs of C57BL/6 mice.

RNA Analysis. RNA extraction and reverse transcription were performed as described (2). Quantitative real-time RT-PCR analysis was performed using a LightCycler and SYBRGreen system (Roche). Data were normalized with the level of GAPDH expression in each sample. Primers for IL12-p35, IL-12p40, IFN-b, GAPDH, and Cxcl10 have been described previously (3). The following primers were used for IL-1b: 5′-GCTTCAGGCAGGCAGTATCAC-3′ and 5′-CGACAGCACGAGGCTTTTT-3′.

Histology. Organs were removed and fixed in 4% paraformaldehyde for 3 days at 4 °C, then embedded in paraffin, sectioned, and stained with H&E or Masson's trichrome. For immunohistochemical staining, tissues were embedded in OCT compound and snap-frozen in liquid nitrogen. Cryostat sections (10 mm thick) were fixed with acetone and methanol, and stained with FITC-conjugated anti-CD45 and biotin-conjugated anti-CD11b antibodies (PharMingen), and subsequently with streptavidin-Alexa Fluor 546 (Molecular Probes) and 4-,6-diamidino-2 phenylindole (DAPI) (Nakarai Tesque). For CVB3 staining, anti-CVB3 antibody (Chemicon) and VECTOR M.O.M. Immunodetection kit (Vector Laboratories) were used. Sections were examined using a fluorescence microscope (Olympus).

1. Niwa H, Yamamura K, Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 108:193–199.
2. Honda K, *et al.* (2005) IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* 434:772–777.
3. Negishi H, *et al.* (2006) Evidence for licensing of IFN-gamma-induced IFN regulatory factor 1 transcription factor by MyD88 in Toll-like receptor-dependent gene induction program. *Proc Natl Acad Sci USA* 103:15136–15141.

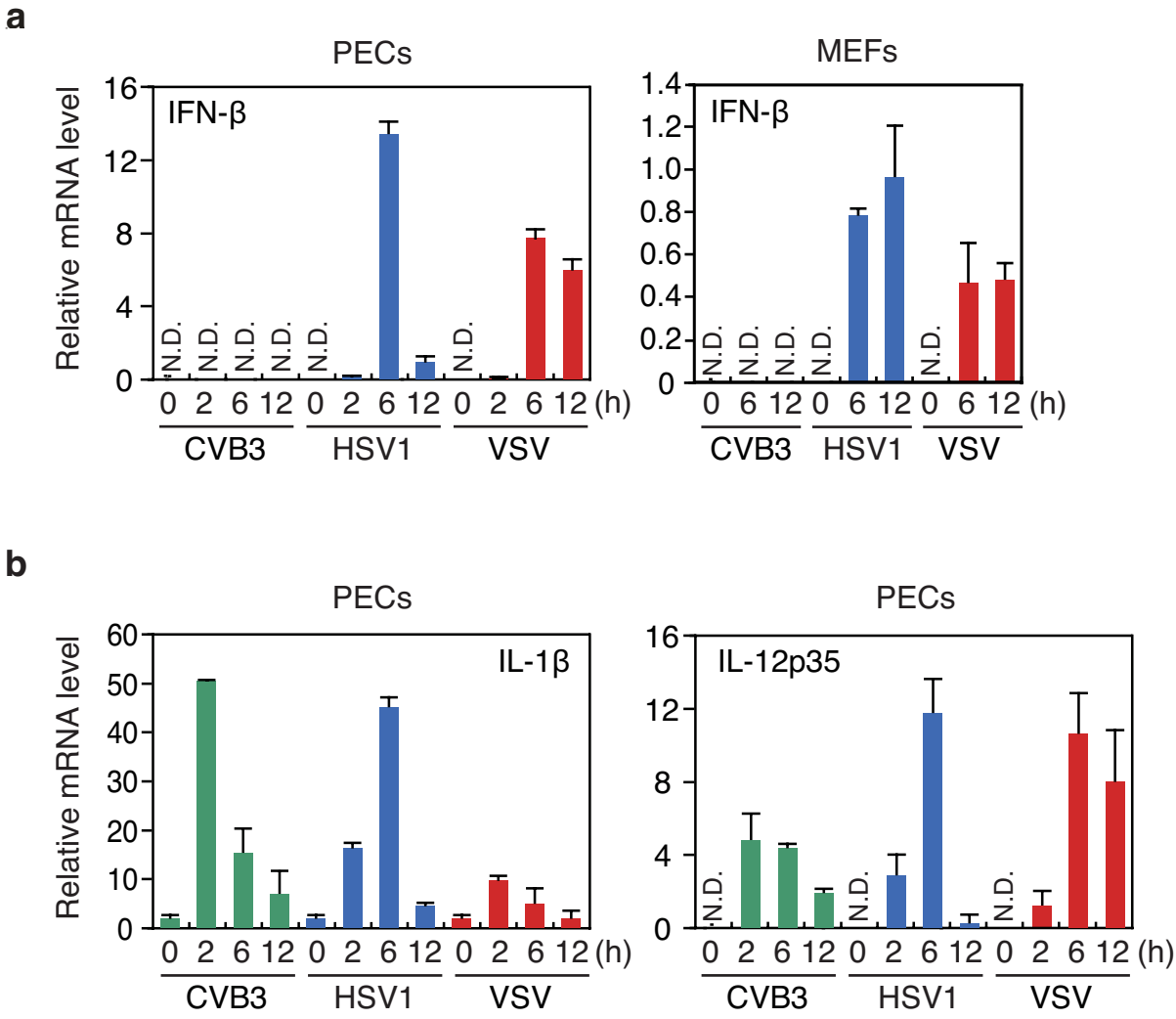

Fig. S1. Type I IFN response in cells infected with CVB3, HSV1 or VSV. (*a*, *b*) PECs or MEFs were infected with CVB3 (3 moi), HSV1 (1 moi), or VSV (1 moi) for the indicated periods. Total RNA was prepared and the mRNA expression levels of the indicated genes were determined by real-time RT-PCR. Data are presented as mean ± SD of triplicate determinations. All experiments were performed more than twice with similar results. ND, not detected.

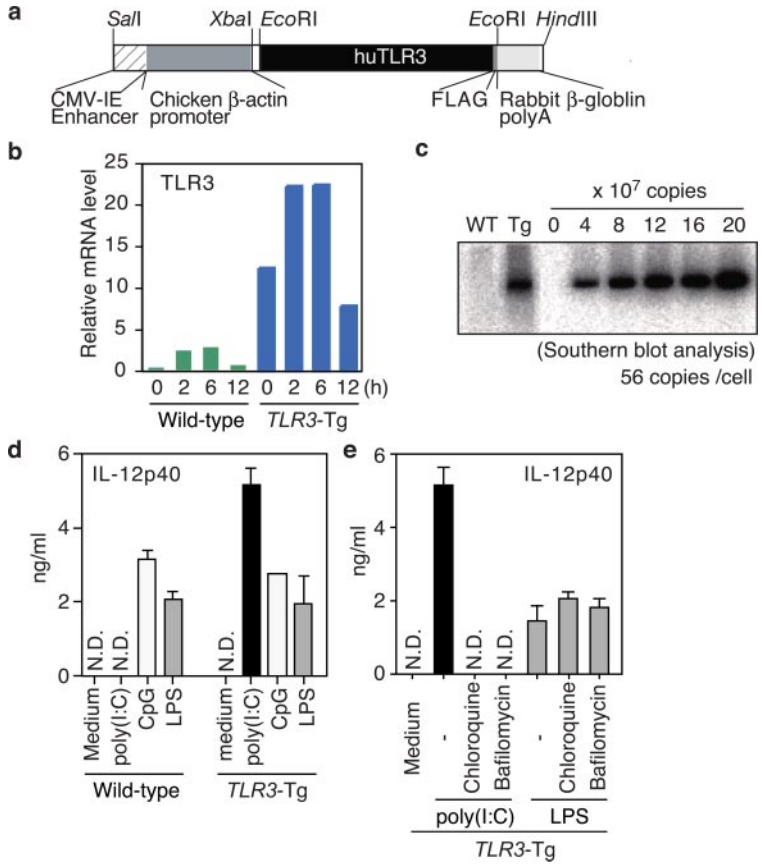

Fig. S2. Generation of transgenic mice. (*a*) Schematic diagram of the DNA fragment containing TLR3 cDNA under the control of the β-actin promoter used for the generation of *TLR3*-Tg mice. (*b*) PECs were stimulated with IFN-β (1000 U/ml) for the indicated periods. Total RNA was prepared and subjected to real-time RT-PCR analysis for TLR3 mRNA expression using primer sets that amplify both human and mouse TLR3 mRNA. The following primers were used: 5′-GATTGGGTCTGGGAACAT-3′ and 5′-GCAAACAGAGTGCATGGT-3′. (*c*) Genomic DNA from the spleen of wild-type (WT) or *TLR3*-Tg (Tg) mice was prepared, digested with SalI and HindIII, and subjected to Southern blot analysis. The transgene copy number (56 copies per cell) was determined by comparing the bands of serially diluted plasmid DNA fragments containing TLR3 cDNA. (*d*) PECs were stimulated with poly(I:C) (100 μg/ml), LPS (100 ng/ml), or CpG-DNA (3 μmol/l) for 24 h. The IL-12p40 concentration in the culture supernatant was assessed by ELISA. Data are expressed as mean $\pm$ SD of triplicate samples. ND, not detected. Note that *TLR3*-Tg cells showed augmented response only to poly(I:C) stimulation. (*E*) PECs were stimulated with medium alone, poly(I:C) (100 μg/ml) or LPS (100 ng/ml) in the presence or absence of chloroquine (5 μg/ml) or bafilomycin (30 nmol/l) for 24 h. The IL-12p40 concentration in the culture supernatant was assessed by ELISA. Data are expressed as mean $\pm$ SD of triplicate samples. ND, not detected. All of the experiments were performed more than twice with similar results.

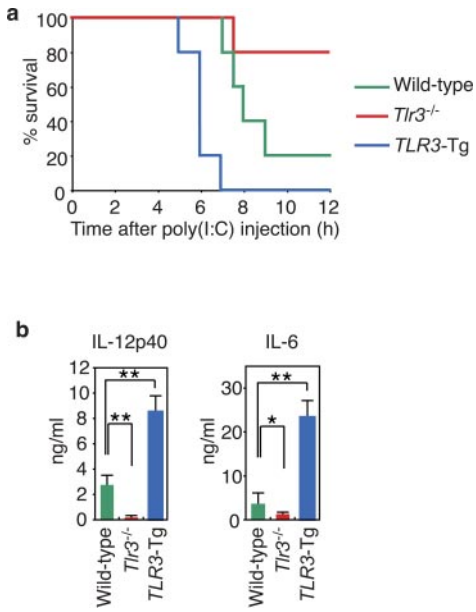

Fig. S3. poly(I:C)-mediated lethal shock in *TLR3*-Tg mice. (a) Age-matched wild-type ($n = 5$), *TLR3*-Tg ($n = 5$), and $Tlr3^{-/-}$ ($n = 5$) mice were injected i.p. with poly(I:C) (10 μg) and D-galactosamine (D-GalN; 10 mg). Survival was monitored for 12 h. (b) Sera were collected 3 h after injection, and the concentration of IL-12p40 and IL-6 in the sera was determined by ELISA. Results represent mean $\pm$ SD of serum samples from five mice. **, $P < 0.01$; *, $P < 0.05$.

a Expression profile of genes in *TLR3*-Tg macrophages stimulated with poly(I:C).

Gene name	Accession No.	Fold increase*
Il1b	NM_008361	48.6
Il6	NM_031168	43.4
Il12b	NM_008352	27.9
Il12a	NM_008351	10.2
Ptgs2	NM_011198	18.1
Hbb-bh1	NM_008219	13.1
Il1a	NM_010554	12.4
Saa1	NM_009117	12.0
Il19	NM_001009940	11.3
Has1	NM_008215	10.2
Cxcl2	NM_009140	10.0
Saa3	NM_011315	8.9
Csf3	NM_009971	8.1
Csf2	NM_009969	7.9
Cxcl1	NM_008176	7.6
Il12rb1	NM_008353	5.4
Il17	NM_010552	5.1
Il10	NM_010548	4.6
Ccl7	NM_013654	4.6
Nfkbiz	NM_030612	3.9
Tnf	NM_013693	3.7

Gene name	Accession No.	Fold increase*
Ifnb1	NM_010510	1.7
Ifit1	NM_008331	1.6
Ifnab	NM_008336	1.6
Ifna1	NM_010502	1.5
Irf1	NM_008390	1.5
Oas1c	NM_033541	1.5
Ifna4	NM_010504	1.3
Irf5	NM_012057	1.3
Ifna9	NM_010507	1.2
Adar	NM_019655	1.2
Ifna5	NM_010505	1.1

*Fold increase over value obtained for wild-type control.

b

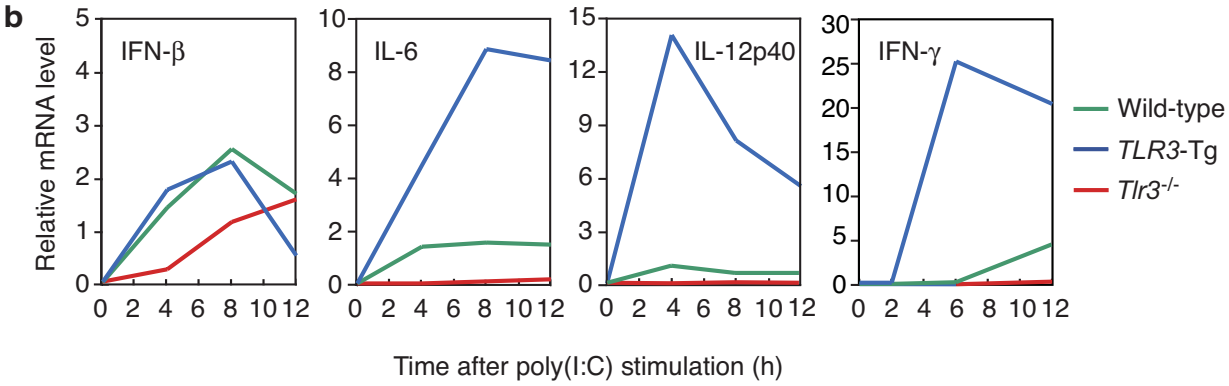

Fig. S4. Expression profile of genes in *TLR3*-Tg macrophages stimulated with poly(I:C). (*a*) Microarray analysis performed on total RNA prepared from wild-type or *TLR3*-Tg PECs stimulated with 100 μg/ml poly(I:C) for 6 h. Shown in the left table are data for genes expressed in *TLR3*-Tg cells that showed 3.7-fold or greater change relative to wild-type cells. Data for type I IFN and its inducible genes are shown in the right table. (*b*) PECs were stimulated with poly(I:C) (100 μg/ml) for the indicated periods. Total RNA was prepared and the mRNA expression levels of the indicated genes were determined by real-time RT-PCR. Note that, although microarray analysis showed that the induction of type II IFN mRNA was not dramatically changed between wild-type and *TLR3*-Tg PECs, real-time RT-PCR analysis revealed that type II IFN mRNA was markedly induced in *TLR3*-Tg PECs stimulated with poly(I:C).

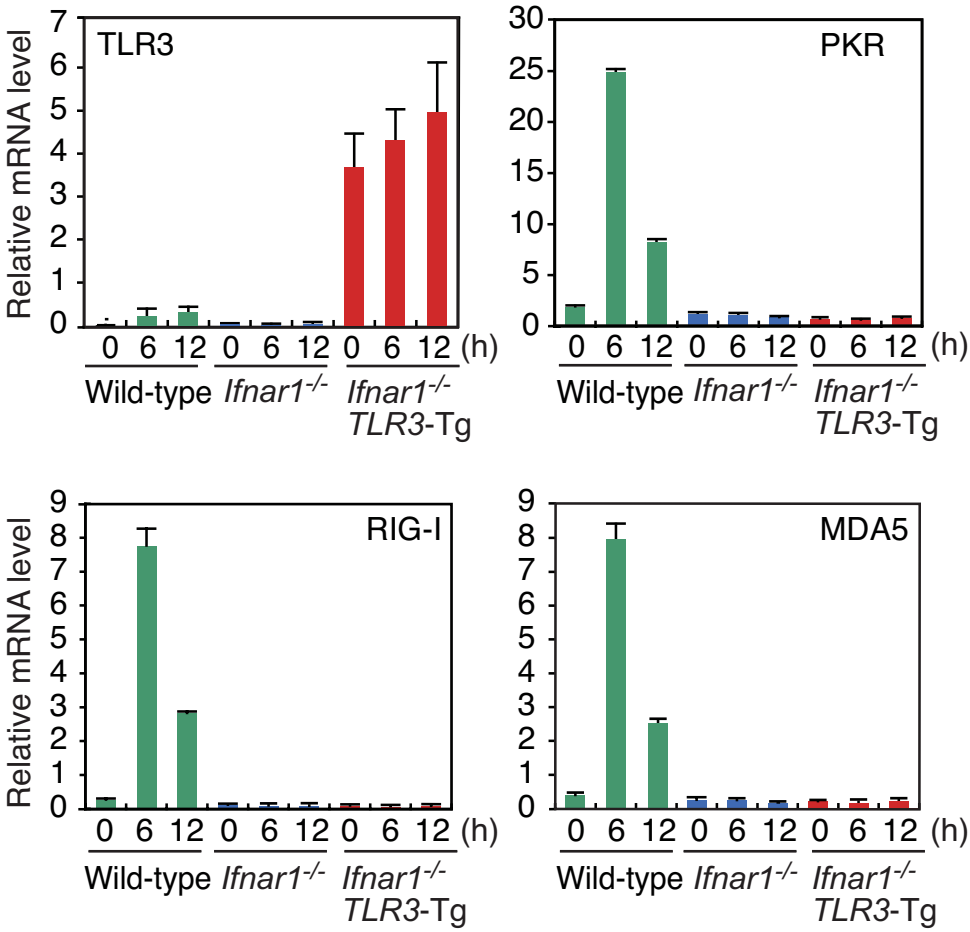

Fig. S5. Expression of mRNAs for dsRNA-recognition molecules in *TLR3*-Tg *Ifnar1*$^{-/[supi]-}$ peritoneal macrophage. PECs from wild-type, *Ifnar*$^{-/[supi]-}$ mice, and *TLR3*-Tg *Ifnar1*$^{-/[supi]-}$ mice were stimulated with recombinant IFN-β (1000 U/ml) for the indicated periods. Total RNA was prepared and the mRNA expression levels of the indicated genes were determined by real-time RT-PCR. Data are presented as mean ± SD of triplicate determinations. The experiment was performed more than twice with similar results.

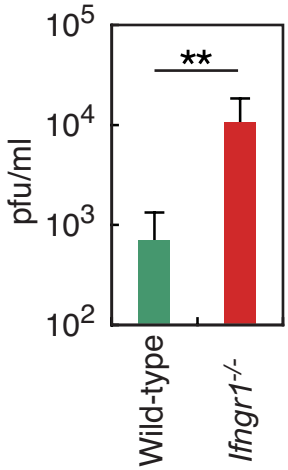

Fig. S6. Virus titer in the sera of CVB3 infected *Ifngr1*$^{-/-}$ mice. Sera from wild-type ($n = 5$) or *Ifngr1*$^{-/-}$ mice ($n = 5$) were collected 2 days after infection, and virus titers were determined by the plaque formation assay. The experiment was performed more than twice with similar results. Results represent mean ± SD of serum samples from five mice. **, $P < 0.01$.

a

VSV (intranasal infection)

	virus positive mice/total (virus titer, pfu/lung)	survival
Wild-type	0/4 (N.D.)	4/4
Tlr3$^{-/-}$	1/4 (7.5±15)	4/4

b

VSV (intraperitoneal infection)

	virus positive mice/total (virus titer, pfu/ml)	survival
Wild-type	0/4 (N.D.)	4/4
Tlr3$^{-/-}$	2/4 (16±23)	4/4

c

HSV1 (corneal infection)

	virus positive mice/total (virus titer, pfu/eyes)	survival
Wild-type	0/4 (N.D.)	4/4
Tlr3$^{-/-}$	3/4 (14±10)	4/4

d

HSV1 (intravenous infection)

	virus positive mice/total (virus titer, pfu/ml)	survival
Wild-type	0/4 (N.D.)	4/4
Tlr3$^{-/-}$	0/4 (N.D.)	4/4

Fig. S7. Sensitivity of *Tlr3*$^{-/-}$ mice to VSV or HSV1 infection. (*a*) Wild-type ($n = 4$) and *Tlr3*$^{-/-}$ mice ($n = 4$) were inoculated intranasally with 5×10^5 pfu of VSV. The lungs from each mouse were collected 5 days after infection and homogenized in 1 ml of RPMI culture medium. Virus titers were determined by plaque formation assay. Another set of mice ($n = 4$ per group), infected by the same method, was monitored for survival over 20 days. (*b*) Wild-type ($n = 4$) and *Tlr3*$^{-/-}$ mice ($n = 4$) were inoculated i.p. with 5×10^6 pfu of VSV and monitored for 20 days. Sera from each mouse were collected 2 days after infection and used to determine virus titers by plaque formation assay. (*c*) Eye corneas of anesthetized wild-type ($n = 4$) and *Tlr3*$^{-/-}$ ($n = 4$) mice were scarified five times with a sterile 25-gauge needle and then infected with 2.5 μl of PBS containing 5×10^5 PFU of HSV1. Eyes from each mouse were collected 6 days after infection, homogenized in 1 ml RPMI culture medium, and assayed for virus titers by plaque formation assay. Another set of mice ($n = 4$ per group) was infected by the same method and then monitored for survival over 20 days. (d) Wild-type ($n = 4$) and *Tlr3*$^{-/-}$ mice ($n = 4$) were inoculated intravenously with 5×10^6 pfu of HSV1 and monitored for 20 days. Sera from each mouse were collected 2 days after infection when sera virus titers were determined by plaque formation assay.