Supporting Information

Kuniyoshi et al. 10.1073/pnas.1401674111

SI Materials and Methods

Reagents and Cells. ELISA kits for mouse IFN-α were from R&D Systems. Amaxa Cell Line Nucleofector Kit R was from Lonza. Bone marrow-derived dendritic cells (DCs) were prepared from the femurs and tibias of mice. Briefly, the cells were cultured in RPMI medium 1640 supplemented with 10% FCS, 100 μM 2-mercaptoethanol, and 10 ng/mL human Flt3 ligand (PeproTech). After 9 d the cells were collected and used as Flt3 ligand-induced plasmacytoid DCs (pDCs).

Generation of *Mex3c*$^{-/-}$ Mice. Genomic DNA containing *Mex3c* was isolated from GSI-I embryonic stem cells and characterized by restriction enzyme mapping and sequence analysis. A targeting vector was designed to replace exon 2 containing the really interesting new gene (RING)-finger domain with a neomycin-resistance gene. A 1.1-kb ClaI–BamHI fragment was used as the 5′ fragment region, and a 6.9-kb SalI–NotI fragment was used as the 3′ homology region. GSI-I embryonic stem cells were electroporated with 40 μg of NotI-linearized vector. After selection with G418, the drug-resistant clones were picked and screened with PCR and Southern blot analysis. These clones were individually microinjected into blastocysts derived from C57BL/6 mice and transferred to pseudopregnant females. Mating chimeric male mice to C57BL/6 female mice resulted in the transmission of the mutant allele to the germ line. The resulting *Mex3c*$^{+/-}$ mice were intercrossed to generate *Mex3c*$^{-/-}$ mice.

FACS Analysis. Antibodies for flow cytometry were purchased from BD Biosciences. Cells were washed in ice-cold magnetic cell sorting (MACS) buffer (2% FCS and 0.02% NaN_3 in PBS), then incubated with each antibody for 30 min and washed twice with FACS buffer. Data were acquired on a FACSCalibur or FACSCanto II flow cytometer (BD Biosciences) and analyzed using FlowJo (TreeStar).

Electroporation. HeLa cells (1×10^6) were electroporated with the indicated plasmids (1μg) by the Nucleofector system (Lonza) to produce cells stably expressing the corresponding plasmid-encoded genes.

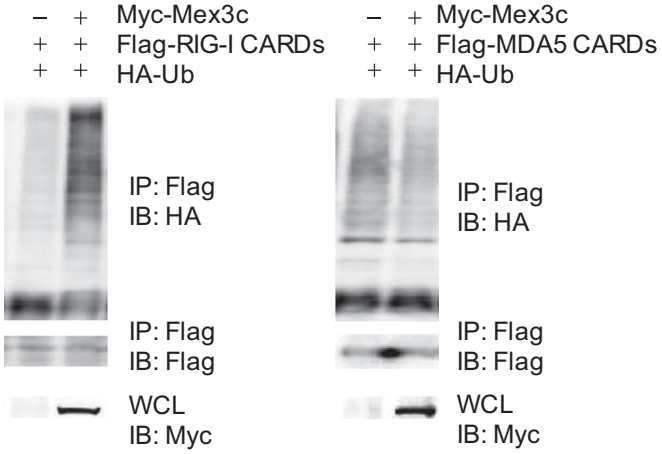

Fig. S1. Enhanced ubiquitination of retinoic acid inducible gene-1 (RIG-I) CARDs by MEX3C. Anti-Flag immunoprecipitates (IP) or whole-cell lysates (WCL) prepared from HEK293 cells transfected with the indicated plasmids were subjected to immunoblot (IB) with the indicated antibodies. Image shown is the representative of three independent experiments.

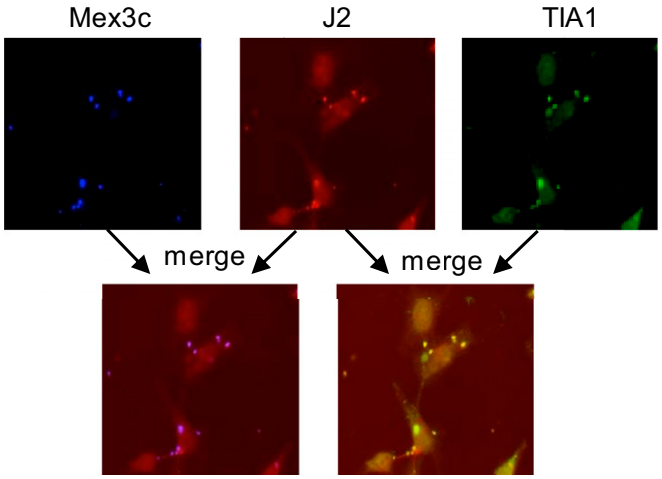

Fig. S2. Colocalization of MEX3C with viral RNA. HeLa cells were electroporated with Flag-MEX3C and infected with Newcastle disease virus (NDV). After 24 h the cells were stained with the indicated antibodies and analyzed by fluorescence microscopy. Image shown is the representative of three independent experiments.

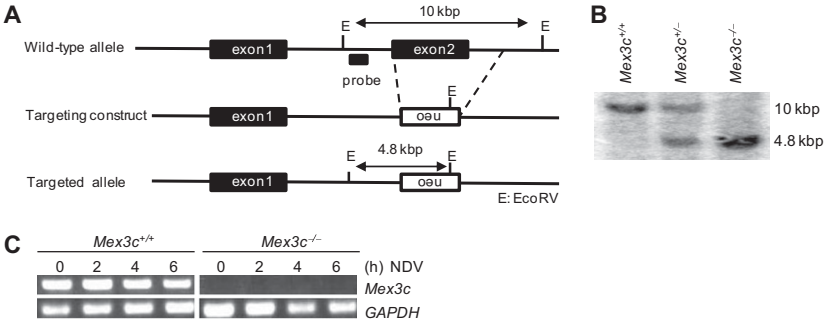

Fig. S3. Generation of *Mex3c*$^{-/-}$ mice. (*A*) Schematic representation of the mouse *Mex3c* gene, the targeting vector, and the targeted allele. The targeting vector was designed by replacing exon 2, containing the RING-finger domain, with a neomycin-resistance gene. E, *Eco*RV. (*B*) Southern blot analysis of offspring from the heterozygote intercrosses. Genomic DNA was extracted from the mouse tails, digested with *Eco*RV, separated by electrophoresis, and hybridized with the radiolabeled probe indicated in *A*. The Southern blot shows a single 10-kb band for the WT mice (*Mex3c*$^{+/+}$), a 4.8-kb band for the homozygous mice (*Mex3c*$^{-/-}$), and both bands for the heterozygous (*Mex3c*$^{+/-}$) mice. (*C*) RT–PCR of RNA from *Mex3c*$^{+/+}$ and *Mex3c*$^{-/-}$ macrophages infected with NDV for the indicated periods.

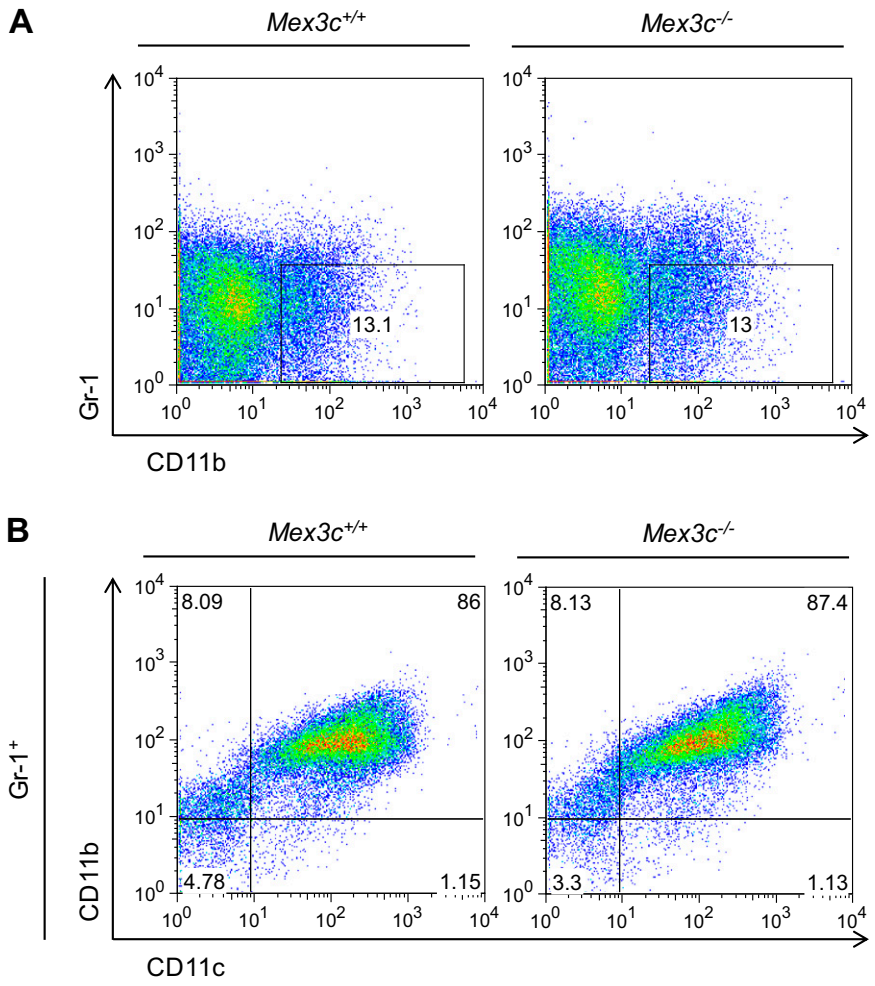

Fig. S4. The population of peritoneal macrophage and conventional DC was comparable between *Mex3c*$^{+/+}$ and *Mex3c*$^{-/-}$ mice. (*A*) Peritoneal macrophages prepared from *Mex3c*$^{+/+}$ and *Mex3c*$^{-/-}$ mice were stained with Gr-1 and CD11b and analyzed by flow cytometry. (*B*) GM-CSF–induced DCs prepared from *Mex3c*$^{+/+}$ and *Mex3c*$^{-/-}$ mice were stained with Gr-1, CD11c, and CD11b and analyzed by flow cytometry. Data are from three independent experiments.

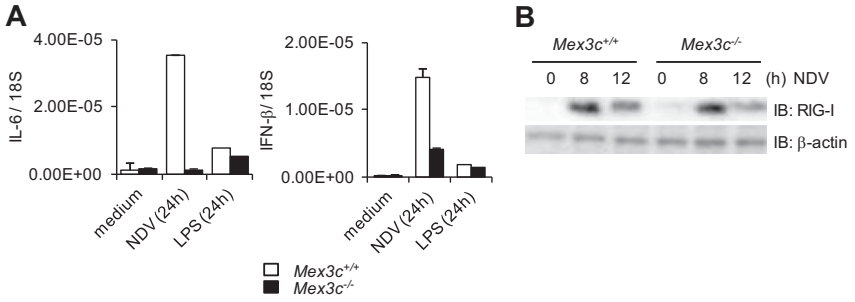

Fig. S5. Impaired cytokine mRNA induction after NDV infection in MEX3C-deficient macrophages. (*A*) Peritoneal macrophages from *Mex3c*$^{+/+}$ and *Mex3c*$^{-/-}$ mice were treated with LPS (100 ng/mL) or NDV [multiplicity of infection (moi) 0.5] for 24 h, and their total RNAs were analyzed with quantitative PCR. Data are from three independent experiments. The results shown are means ± SD ($n = 3$). (*B*) Peritoneal macrophages from *Mex3c*$^{+/+}$ and *Mex3c*$^{-/-}$ mice were infected with NDV (moi 0.5) for the indicated periods, and the protein levels of RIG-I and β-actin were determined with immunoblot analysis.

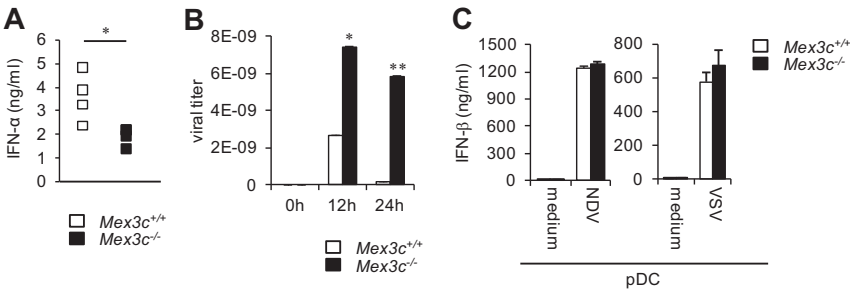

Fig. S6. Cytokine production of *Mex3c*-deficient mice against RNA viruses. (*A*) *Mex3c*$^{+/+}$ and *Mex3c*$^{-/-}$ mice were infected with Japanese encephalitis virus (JEV) for 5 d, and the concentrations of IFN-α in their sera were measured with ELISA. Data are from three independent experiments. The results shown are means ± SD ($n = 3$). *$P < 0.05$ and **$P < 0.005$ compared with controls. (*B*) Peritoneal macrophages from *Mex3c*$^{+/+}$ and *Mex3c*$^{-/-}$ mice were infected with NDV for the indicated periods. The concentrations of viral RNAs in the supernatants were measured with quantitative PCR. Data are from three independent experiments. The results shown are means ± SD ($n = 3$). *$P < 0.05$ and **$P < 0.005$ compared with controls. (*C*) pDCs prepared from *Mex3c*$^{+/+}$ and *Mex3c*$^{-/-}$ bone marrow were infected with NDV (moi 0.5) or vesicular stomatitis virus (VSV) (moi 3). The concentration of IFN-β in the supernatant was measured with ELISA. Data are from three independent experiments. The results shown are means ± SD ($n = 3$).