Supporting Information

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SI Materials and Methods

VSV Infection and Quantification. Infection was carried out by seeding 5×10^5 MEF cells per well in a 6-well plate and allowing 12 h for cells to adhere. One representative well per cell type was enumerated for calculation of MOI and an inoculum of virus in 500 mL DMEM was added to each well for 1 h at 37 °C. The inoculum was removed and replaced with complete medium for the remainder of the indicated incubation time. Virus titers were measured by a standard plaque assay on Vero cells. Briefly, 18 h and 24 h after infection, the culture supernatants were recovered and kept at -80 °C. After the addition of the supernatants, the cells were overlaid with DMEM containing 1% methyl cellulose and 5% FCS and were incubated for 48 h. After staining with crystal violet, the number of plaques was counted.

Primers Used for Quantitative PCR. mIFNα4 forward, CTGCTACTTGGAATGCAACTC; mIFNα4 reverse, CAGTCTTGCCAGCAAGTTGG; mIFNβ forward, GCACTGGGTGGAATGAGACTATTG; mIFNβ reverse, TTCTGAGGCATCAACTGACAGGTC; mIL-6 forward, CCTCTCTGCAAGAGACTTCCATCCAGTTGC; mIL-6 reverse, GACTATTTTATGTAAATCTTTTACCTCTTGGTTGAAG.

Preparation of Primary Macrophages. Primary macrophages were prepared from Atg5$^{+/+}$ or Atg5$^{-/-}$ neonatal liver. Briefly, isolated single liver cells from less than 24 h old pups were cultured with macrophage CSF-conditioned RPMI media including 30% macrophage CSF supernatant from L929 cell line culture and 10% heat-inactivated FBS, 1% Hepes, and 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco) for 7 days. At day 4, media was replaced with fresh conditioned media. Genotypes were screened by PCR from tails of pups as described previously (9). Atg5$^{+/+}$ and Atg5$^{-/-}$ macrophage phenotype was confirmed by FACS (data not shown).

Quantification of Mitochondrial DNA by PCR. The PCR protocol consisted of 50 °C for 2 min and 95 °C for 10 min, then 40 cycles of 95 °C for 15 seconds and 60 °C annealing/extension for 1 min, with real-time data collection. Primers used to amplify mouse mtDNA were as follows: 18S 1546, forward, 5′-TAGAGGGACAAGTGGCGTTC-3′; 18S 1650, reverse, 5′-CGCTGAGCCAGTCAGTGT-3′; and mouse COI, forward, 5′-GCCCCAGATATAG-CATTCCC-3′; mouse COI, reverse, 5′-GTTCATCCTGTTCCTGCTCC-3′.

Southern Blotting for Mitochondrial DNA. Briefly, 2 μg of total DNA was digested with ScaI overnight, electrophoresed on a 0.7% agarose gel, and transferred to a Hybond-N membrane (GE Healthcare). Isolated mtDNA was subsequently probed with a mouse mtDNA probe spanning nucleotides 5556 to 6268 of mouse mtDNA (generated using primers mtDNAprob-F GGCTTTGGAAACTGACTTGT and mtDNAprob-R TTGCGATAATTATAGTGGCT). Nuclear DNA was quantified by hybridizing to an 18s rDNA probe (generated using primers m18s SB-F TACCTGGTTGATCCTGCCA, m18s SB-R AAAGTGGACTCATTCCAATT). The p32 signal was measured by a Kodak X-Omat 200A processor.

Western Blot Analysis. Following 12 h of Poly I:C stimulation, cells were lysed in RIPA lysis buffer [200 mM Tris-HCl (pH 7.5) containing 150 mM NaCl, 1% Nonidet P-40], in the presence of protease inhibitor mixture (Roche). Samples were run on an SDS/PAGE (7.5%) under reducing conditions and subsequently blotted using the indicated antibodies: Atg5 (anti-atg5, Cosmo Bio), p62 (autophagy SQSTM1, Abgent), Cardif (polyclonal AT107, Alexis), Apoptosis inducing factor (sc-13116, Santa Cruz Biotechnology), and β-actin (monoclonal anti-β-actin, Sigma).

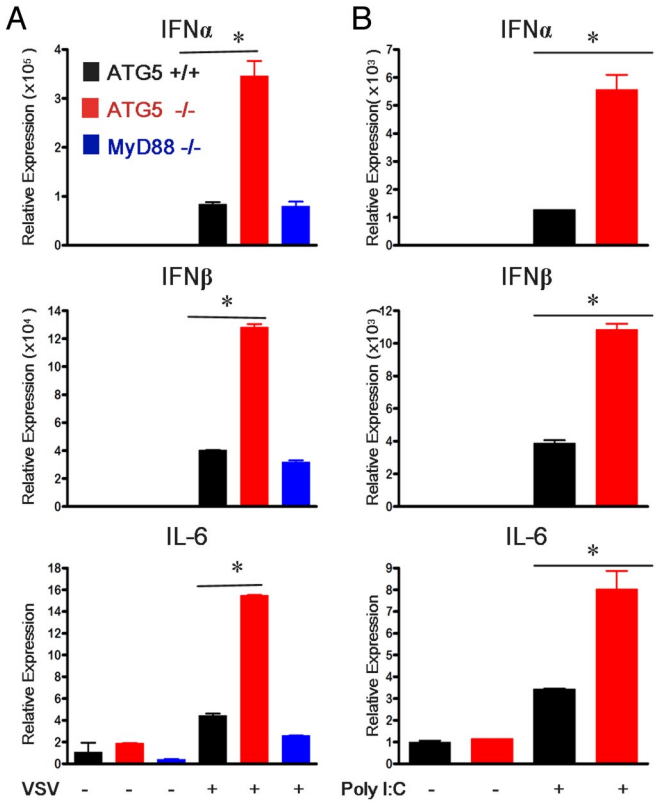

Fig. S1. Atg5-deficient MEFs show increased cytokine production to Poly I:C and VSV stimulation. Atg5$^{+/+}$ Atg5$^{-/-}$ and MyD88$^{-/-}$ MEFs were incubated with VSV-GFP (at a multiplicity of infection of 4, *A*) or transfected with 1 μg/mL Poly I:C (*B*). Twelve hours later, IFNα, IFNβ, and IL-6 production were assessed by measuring mRNA levels by RT-qPCR. *$P < 0.05$, **$P < 0.005$. Data are representative of 5 similar experiments.

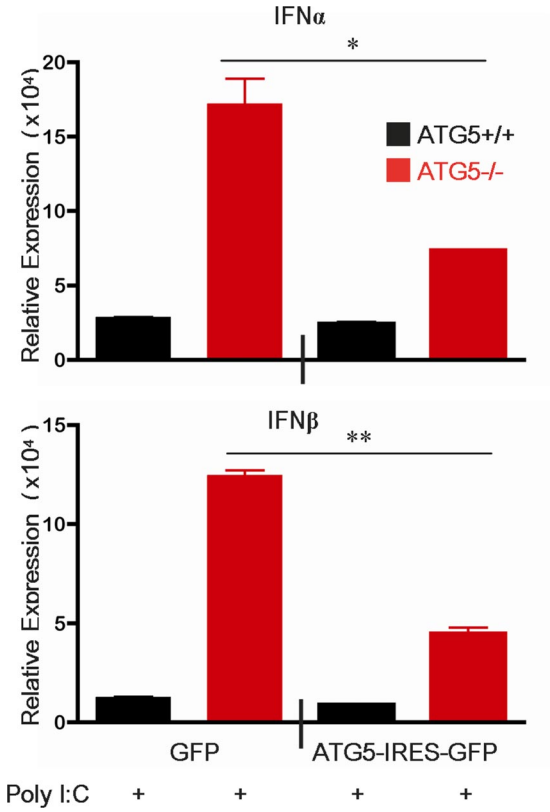

Fig. S2. Atg5 complementation of knockout MEFs. Atg5$^{+/+}$ and Atg5$^{-/-}$ MEFs were transduced with Atg5-IRES-GFP or GFP (control) lentivirus. Only cells with high expression of GFP were sorted by FACS and stimulated with Poly I:C. IFNα and IFNβ production was assessed by measuring mRNA levels by RT-qPCR. *$P <$ 0.05, **$P < 0.005$. Data are representative of 2 similar experiments.

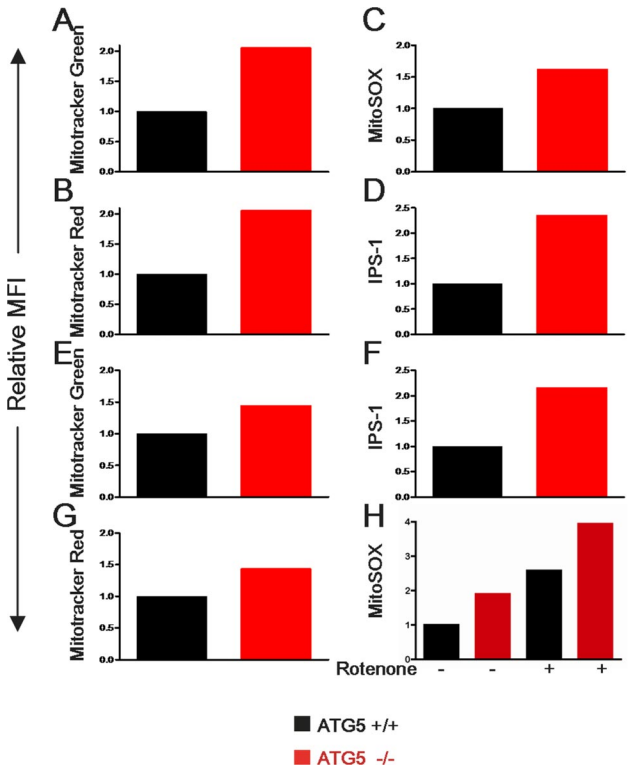

Fig. S3. Relative mean fluorescence intensity comparisons. Unstimulated (*A*-*D*) or rotenone-treated (*H*) $Atg5^{+/+}$ and $Atg5^{-/-}$ MEFs were labeled with MitoTracker Green (*A*), MitoTracker Red (*B*), MitoSOX (*C*), or intracellular staining for IPS-1 (*D*) as described in Fig. 2 (*A* and *B*), Fig. 3 (*D*), or Fig. 4 (*C*) in the main text. Data are representative of 4 similar experiments. Neonatal primary macrophages were labeled with MitoTracker Green (*E*), MitoTracker Red (*G*), and intracellular staining for IPS-1 (*F*).

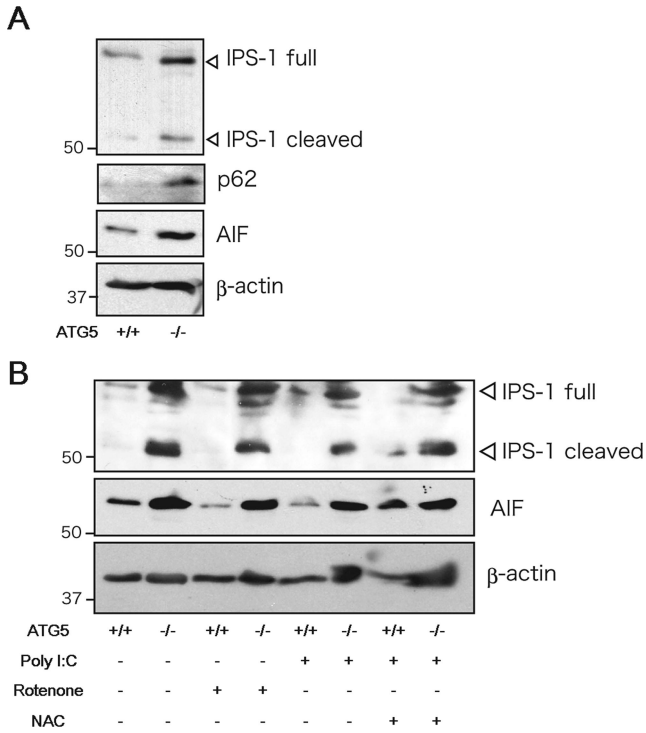

Fig. S4. IPS-1 protein analysis by Western blot. (*A*) Unstimulated Atg5$^{+/+}$ and Atg5$^{-/-}$ MEFs were lysed, and the protein levels of IPS-1, p62, mitochondrial marker (apoptosis-inducing factor), and β-actin were measured by Western blot. (*B*) Unstimulated, Poly I:C-treated, rotenone-treated, or Poly I:C and NAC-treated Atg5$^{+/+}$ and Atg5$^{-/-}$ MEFs were lysed after 12 h of incubation, and lysates were run on SDS/PAGE and blotted with the indicated antibodies. Data are representative of 2 similar experiments.

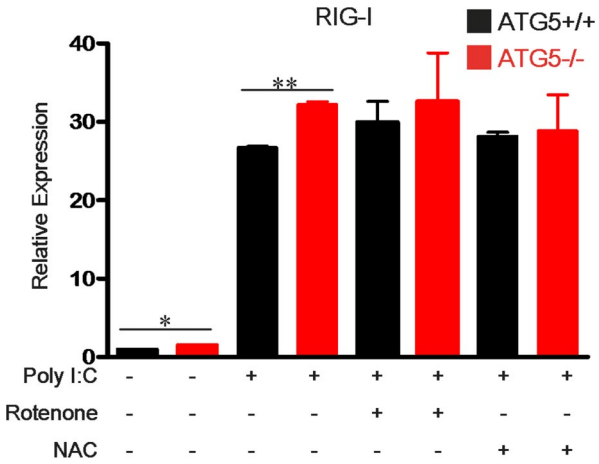

Fig. S5. RIG-I mRNA levels in Atg5$^{+/+}$ and Atg5$^{-/-}$ MEFs. Levels of RIG-I mRNA were measured from unstimulated, Poly I:C-treated, Poly I:C and rotenone-treated, or Poly I:C and NAC-treated Atg5$^{+/+}$ and Atg5$^{-/-}$ MEFs after 12 h of incubation by RT-qPCR. *$P < 0.05$, **$P < 0.005$. Data are representative of 2 similar experiments.