Supplemental Figure 1

Supplementary Figure 1. FBXW7-deficient mice are more sensitive to RNA virus infection by producing more type I interferon. (a) Q-PCR analysis of FBXW7 expression in THP-1 cells transfected for 36 hr with 60nM FBXW7 siRNA (siFBXW7). (b) Q-PCR analysis of TNF-α and IL-6 expression in THP-1 cells transfected for 36 hr with siFBXW7 and infected with RSV. (c) PCR analysis of “floxed” and WT alleles (497 and 315 bp, respectively) using primer B and C, and determination of excision of exons 5 and 6 by primers A and C, which amplify 662 bp in the Lysm$^+$FBXW7$^{flf}$ macrophages and DCs. (d) Q-PCR analysis of FBXW7 expression in peritoneal macrophages, BMDM and DCs. (e) Western blot analysis of FBXW7 protein in macrophages. (f) Flow cytometry analysis of CD11c$^+$ DC, CD11b$^+$Gr-1$^+$ granulocytes and F4/80$^+$CD11b$^+$ macrophages in spleen from FBXW7$^{flf}$ and Lysm$^+$FBXW7$^{flf}$ mice. (g) Statistical analysis of percentages of DCs, macrophages and granulocytes in spleen from FBXW7$^{flf}$ and Lysm$^+$FBXW7$^{flf}$ mice by flow cytometry (n=6 per group). Data are mean ± s.e.m and are representative of three independent experiments. Student’s $t$ test was used for statistical calculation. **$P<0.01$, ***$P<0.001$. 

1
Supplementary Figure 2. FBXW7 inhibits VSV infection and increases type I interferon production in vivo. (a) Survival assay of ~7-week-old FBXW7^{f/f} and Lysm^{f/f}FBXW7^{f/f} mice infected with H1N1 virus (2×10^{3} pfu) by intranasal inoculation. n=9 per group. Kaplan-Meier survival curves were generated and analyzed. (b) Lung histology of FBXW7^{f/f} and Lysm^{f/f}FBXW7^{f/f} mice in response to H1N1 virus. Scale bar, 50 μm. Hematoxylin and eosin staining of lung tissues from mice infected with H1N1 (2×10^{3} pfu) by intranasal inoculation for 6 days. (c) Q-PCR analysis of Flu A expression in organs from FBXW7^{f/f} and Lysm^{f/f}FBXW7^{f/f} mice in (b) was shown. (d) ELISA assay of cytokine production in sera from FBXW7^{f/f} and Lysm^{f/f}FBXW7^{f/f} mice in (b). (e) Q-PCR analysis of IFN-β expression in spleen and lung from FBXW7^{f/f} and Lysm^{f/f}FBXW7^{f/f} mice in (b). (f) ELISA assay of cytokine production in sera from FBXW7^{f/f} and Lysm^{f/f}FBXW7^{f/f} mice (n=6 per group) intraperitoneally injected with HSV. Data are mean ± s.e.m and are representative of three independent experiments. Student’s t test was used for statistical calculation. *P<0.05; **P<0.01; ***P<0.001.
Supplementary Figure 3

FBXW7 inhibits RNA virus infection and promotes type I interferon production in vitro. (a) Q-PCR analysis of IFN-β, IFN-α4 mRNA expression in BMDMs from FBXW7^{f/f} and Lysm^FBXW7^{f/f} mice. Cells were infected with VSV or H1N1 virus. (b) Q-PCR analysis of IFN-β, IFN-α4 mRNA expression in FBXW7^{f/f} and Lysm^FBXW7^{f/f} peritoneal macrophages transfected with LMW-poly (I:C) by Jetprime for 3, 6 hr. (c) Immunoblot analysis of the knockdown of exogenous FBXW7 in peritoneal macrophages. (d) Q-PCR analysis of IFN-β, IFN-α4 mRNA expression in FBXW7-silenced peritoneal macrophages infected with VSV or H1N1 virus. (e) Q-PCR analysis of VSV-G mRNA expression in FBXW7-silenced macrophages infected with VSV-GFP at a MOI of 0.1, 1 or 10. (f) Flow cytometry analysis of GFP^+ cells in FBXW7-silenced macrophages infected with VSV-GFP. (g) Q-PCR analysis of IFN-β mRNA in FBXW7-overexpressed HEK293T cells infected with VSV at indicated MOI and time points. (h) Flow cytometry analysis of GFP^+ in HEK293T cells overexpressing FBXW7 infected with VSV-GFP for 12 hr. (i) Q-PCR analysis of VSV-G mRNA in FBXW7-overexpressed HEK293T cells infected with VSV at indicated MOI and time points. Data are mean ± s.e.m and are representative of three independent experiments. Student’s t test was used for statistical calculation. *P<0.05; **P<0.01; ***P<0.001.
Supplementary Figure 4

**Supplementary Figure 4.** FBXW7 positively regulates RIG-I signal pathway through protecting RIG-I from degradation. (a) Immunoblot analysis of phosphorylated or total proteins in lysates of FBXW7<sup>f/f</sup> and Lysm<sup>+</sup>FBXW7<sup>f/f</sup> peritoneal macrophages transfected for indicated hours with LMW-poly (I:C). (b) Immunoblot analysis of RIG-I in lysates of FBXW7<sup>f/f</sup> and Lysm<sup>+</sup>FBXW7<sup>f/f</sup> macrophages transfected with LMW-poly (I:C). (c) Immunoblot analysis of MAVS in lysates of FBXW7<sup>f/f</sup> and Lysm<sup>+</sup>FBXW7<sup>f/f</sup> macrophages infected for indicated hours with VSV. (d) Immunoblot analysis of MDA5 in lysates of FBXW7<sup>f/f</sup> and Lysm<sup>+</sup>FBXW7<sup>f/f</sup> macrophages infected for indicated hours with EMCV. (e) Immunoblot analysis of MDA5 in lysates of FBXW7<sup>f/f</sup> and Lysm<sup>+</sup>FBXW7<sup>f/f</sup> macrophages transfected for indicated hours with HMW-poly (I:C). (f) Immunoblot analysis of MDA5 in lysates of FBXW7<sup>f/f</sup> and Lysm<sup>+</sup>FBXW7<sup>f/f</sup> peritoneal macrophages treated with CHX (40 μg/ml) for indicated hours after infection with EMCV for 1 hr. (g) Quantification of relative MDA5 levels is shown in the right panel. (h) Q-PCR analysis of IFN-β, IFN-α4 mRNA expression in FBXW7<sup>f/f</sup> and Lysm<sup>+</sup>FBXW7<sup>f/f</sup> peritoneal macrophages infected with EMCV. Data are mean ± s.e.m and are representative of three independent experiments. Student’s t test was used for statistical calculation. ns, not significant.
Supplementary Figure 5.

(a) Confocal microscopy images of HEK293T cells transfected with Flag-FBXW7 and infected for indicated hours with VSV and labeled with antibodies to the appropriate protein. Scale bar, 20 μm. (b) Confocal microscopy images of HEK293T cells transfected with Flag-FBXW7 and pretreated with 30 μM Leptomycin B (LMB) for 4 hr, then infected for indicated hours with VSV and labeled with antibodies to the appropriate protein. Scale bar, 20 μm. (c) Confocal microscopy images of FBXW7fl/fl and Lysm+/−FBXW7fl/fl BMDM infected for indicated hours with VSV. Scale bar, 20 μm. (d) Sequence alignment of FBXW7 shows nuclear export signal (NES) (Φ-x2-3-Φ-x2-3-x-Φ-x-Φ, "Φ" is a hydrophobic residue and "x" is any other amino acid). (e) Flow cytometry analysis of GFP in HEK293T cells overexpressed FBXW7 (wt) or FBXW7 (mutant) and infected with VSV-GFP for 12 hr. (f) Coimmunoprecipitation and immunoblot analysis of HEK293T cells transfected for 24 hr with Flag-FBXW7 and infected with VSV. (g) Immunoblot analysis of HEK293T cells transfected for 24 hr with Flag-FBXW7 (wt) or Flag-FBXW7 (mutant) and infected with VSV.
Supplementary Figure 6. **FBXW7 interacts with SHP2.** (a) The information of SHP2 in FBXW7-interacting proteins identified by mass spectrometry. (b) Confocal microscopy imaging of HEK293T cells transfected with Flag-FBXW7 and Myc-SHP2 for 24 hr, then infected for indicated hours with VSV and labeled with antibodies to the appropriate protein. Scale bar, 10 μm.
Supplementary Figure 7

(a) Q-PCR analysis of SHP2 mRNA expression in FBXW7\textsuperscript{fl/fl} and Lysm\textsuperscript{-}/FBXW7\textsuperscript{fl/fl} peritoneal macrophages infected for indicate hours with VSV. Data are mean ± s.e.m and are representative of three independent experiments. Student’s t test was used for statistical calculation. ns, not significant. (b) Immunoblot analysis of SHP2 in lysates of FBXW7-silenced RAW 264.7 cells infected with VSV. (c, d) Immunoblot analysis of SHP2 in lysates of FBXW7-silenced macrophages treated with CHX (40 μg/ml) for indicated hours after infection with VSV for 1 hr. The protein level of SHP2 was shown in the right panel, which was relative to β-actin protein. (e) Immunoblot analysis of the K-48 ubiquitination of SHP2 in HEK293T cells cotransfected with Flag-SHP2, HA-ub, along with increasing concentrations (wedge) of vectors for the Myc-FBXW7 constructs and treated with MG132 before cell harvest. (f) Immunoblot analysis of the K-48 ubiquitination of SHP2 in HEK293T cells overexpressing FBXW7 and infected with VSV and treated with MG132 before cell harvest. (g) Immunoblot analysis of the ubiquitination of SHP2 in HEK293T cells cotransfected with Flag-SHP2, HA-ub, along with Myc-FBXW7 or Myc-∆FBOX vector and treated with MG132 before cell harvest.

Supplementary Figure 7. FBXW7 mediates the degradation and ubiquitination of SHP2. (a) Q-PCR analysis of SHP2 mRNA expression in FBXW7\textsuperscript{fl/fl} and Lysm\textsuperscript{-}/FBXW7\textsuperscript{fl/fl} peritoneal macrophages infected for indicate hours with VSV. Data are mean ± s.e.m and are representative of three independent experiments. Student’s t test was used for statistical calculation. ns, not significant. (b) Immunoblot analysis of SHP2 in lysates of FBXW7-silenced RAW 264.7 cells infected with VSV. (c, d) Immunoblot analysis of SHP2 in lysates of FBXW7-silenced macrophages treated with CHX (40 μg/ml) for indicated hours after infection with VSV for 1 hr. The protein level of SHP2 was shown in the right panel, which was relative to β-actin protein. (e) Immunoblot analysis of the K-48 ubiquitination of SHP2 in HEK293T cells cotransfected with Flag-SHP2, HA-ub, along with increasing concentrations (wedge) of vectors for the Myc-FBXW7 constructs and treated with MG132 before cell harvest. (f) Immunoblot analysis of the K-48 ubiquitination of SHP2 in HEK293T cells overexpressing FBXW7 and infected with VSV and treated with MG132 before cell harvest. (g) Immunoblot analysis of the ubiquitination of SHP2 in HEK293T cells cotransfected with Flag-SHP2, HA-ub, along with Myc-FBXW7 or Myc-∆FBOX vector and treated with MG132 before cell harvest.
Supplementary Figure 8. Interaction of FBXW7 with RIG-I and SHP2. (a) Immunoblot analysis of SHP2 expression in SHP2$^{+/+}$ and Lysm$^{+/+}$ peritoneal macrophages. (b) Immunoblot analysis of SHP2 and Flag-FBXW7 expression in SHP2$^{+/+}$ and Lysm$^{+/+}$ peritoneal macrophages. (c) Immunoblot analysis of RAW264.7 infected with VSV or stimulated with LMW-poly (I:C) for indicate hours, followed by immunoprecipitation with SHP2-conjugated agarose or immunoglobulin G (IgG)-conjugated agarase. (d) Coomassie brilliant blue staining of purified Flag-FBXW7, Myc-RIG-I, HA-SHP2 tag-proteins from HEK293T cells transfected with Flag-FBXW7, Myc-RIG-I, HA-SHP2 respectively and infected with VSV for 6 hours. (e) The purified fusion protein of Flag-FBXW7 was mixed and incubated with Myc-RIG-I or/and HA-SHP2 protein, followed by immunoprecipitation with Flag-M2 beads. Here shows the result of Immunoblot and Coomassie brilliant blue staining of Flag-FBXW7, Myc-RIG-I, HA-SHP2 tag-protein interaction in vitro.
Supplementary Figure 9. Uncropped scans of the western blots related to Figure 3-5
Supplementary Figure 10

**Supplementary Figure 10.** Uncropped scans of the western blots related to Figure 6-7
Supplementary Figure 11

Supplementary Figure 11. Uncropped scans of the western blots related to Supplementary Figure 1-7
Supplementary Figure 12

Supplementary Figure 12. Uncropped scans of the western blots related to Supplementary Figure 8
Supplementary Table 1. PCR Primers for expressing vectors, related to experimental procedures

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<th>Reverse Primer</th>
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Supplementary Table 2. RNA quantification, related to experimental procedures

Mouse FBXW7 forward primer, 5’-TGCAAGTCTCAGATTATACC-3’;
Mouse FBXW7 reverse primer, 5’-ACTTCTCTGGTCCGCTCCAGC-3’;
Mouse SHP2 forward primer, 5’-AGGTCGGACAAGGAAACACA-3’;
Mouse SHP2 reverse primer, 5’-CCCTCATTTCGTACAGGGT-3’;
Mouse IFN-β forward primer, 5’-ATGAGTGTTGTTGACAGGC-3’;
Mouse IFN-β reverse primer, 5’-TGACCTTTCACAGTAGATTCA-3’;
Mouse IFN-α4 forward primer, 5’-TACTCAGCAGACCTTAACCT-3’;
Mouse IFN-α4 reverse primer, 5’-CAGATTTGGCAGGACTTTGAC-3’;
Mouse IL-6 forward primer, 5’-TAGTCCTTCTACTCCCATTTCC-3’;
Mouse IL-6 reverse primer, 5’-TTGGTCTTTAGCCACTCTTC-3’;
Mouse RIG-I forward primer: 5’-CAGATCCGAGACACTAAAGGGA-3’;
Mouse RIG-I reverse primer: 5’-TCCTCAGCCTTGTCTTCA-3’
VSV forward primer, 5’-ACGGCGTACTTCCAGATGG-3’;
VSV reverse primer, 5’-CCTCGGTTCAGACATCCAGGT-3’;
Mouse GAPDH forward primer, 5’- AACCTTTGGCATTGTTGGAAGG-3’;
Mouse GAPDH reverse primer, 5’-ACACATTGGGGGTAGGAACA-3’;
Human FBXW7 forward primer, 5’-GACGCGAATTACATCTGTC-3’;
Human FBXW7 reverse primer, 5’-GTAGCAGGTCTTTGGGTTC-3’;
Human IFN-β forward primer, 5’-CATTACCTGAAGGCAAGGA-3’;
Human IFN-β reverse primer, 5’-CATTGTCCAGTCAGAGAGG-3’;
Human GAPDH forward primer, 5’-TCAAGAAGGTGTGAAGCAG-3’;
Human GAPDH reverse primer, 5’-GAGGGGAGATTCAGTGTGGT-3’