Supporting Information

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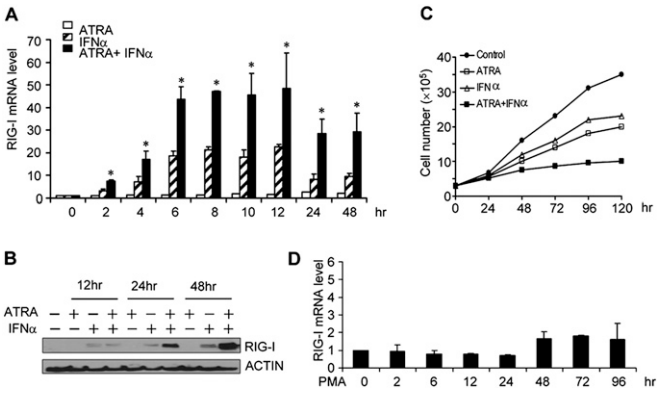

Fig. S1. ATRA and IFN-α synergistically induce RIG-I expression and inhibit cell proliferation in U937 cells. (*A* and *C*) U937 cells were treated with 1 μM ATRA and/or 1,000 U/mL IFN-α for different time courses. RIG-I mRNA level (*A*) and protein level (*B*) were measured by real-time PCR (n = 3, mean ± SD, RA + IFN-α group vs. IFN-α group; $*P < 0.05$) and Western blotting assays, respectively. (*C*) Cell numbers were counted at the indicated time points after the treatments. (*D*) U937 cells were treated with 1 nM phorbol 12-myristate 13-acetate (PMA) for the different time points as indicated, and RIG-I mRNA level was measured by real-time PCR (n = 3, mean ± SD).

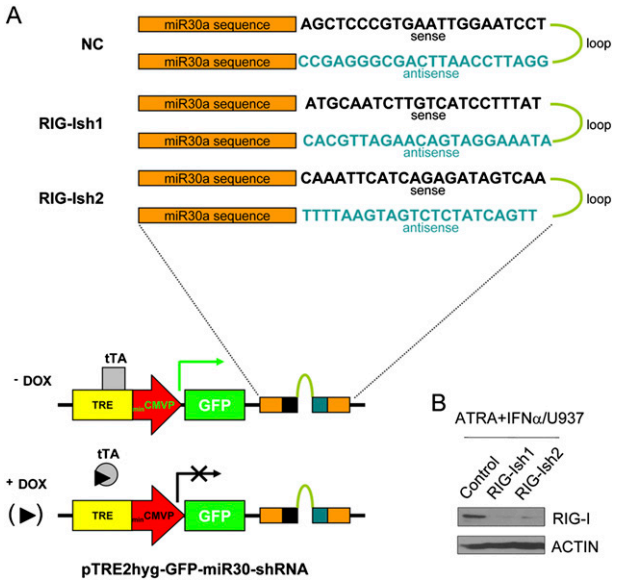

Fig. S2. Establishment of RIG-I knockdown U937 cell lines. (*A*) (*Upper*) Core shRNA sequences are listed. The core shRNA sequences and the flanked miR30a sequences are inserted into the 3′ UTR of a GFP-coding sequence in pTRE2hyg vector. (*Lower*) Production of GFP-shRNA transcript in host cells is under the control of a tetracycline-controlled transactivator (tTA)-inducible tetracycline-responsive element CMV promoter (TRE-CMVP) regulatory element. (*B*) After the stimulation with 1 μM ATRA plus 1,000 U/mL IFN-α2b for 48 h, RIG-I protein expression levels in the control or RIG-I knockdown U937 cell lines (RIG-Ish1 and RIG-Ish2) were assessed by Western blotting assay.

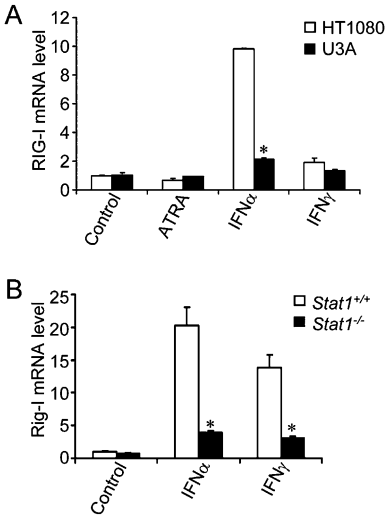

Fig. S3. IFN-α stimulates RIG-I expression through STAT1. (*A*) STAT1 WT HT1080 cells or STAT1-deficient derivative U3A cells (1, 2) were individually treated without or with 1 μM ATRA, 1,000 U/mL IFN-α, or 1,000 U/mL IFN-γ for 12 h. The mRNA levels of RIG-I were determined by real-time PCR assay (n = 3, mean ± SD; *$P < 0.05$). (*B*) BM myeloid cells from Stat1 WT or knockout mice were treated with 1,000 U/mL IFN-α or 30 ng/mL IFN-γ for 2 h, and the Rig-I mRNA levels were then measured by real-time PCR assay (n = 3, mean ± SD; *$P < 0.05$).

1. DeVries TA, Kalkofen RL, Matassa AA, Reyland ME (2004) Protein kinase Cdelta regulates apoptosis via activation of STAT1. *J Biol Chem* 279:45603–45612.
2. Müller M, et al. (1993) Complementation of a mutant cell line: Central role of the 91 kDa polypeptide of ISGF3 in the interferon-alpha and -gamma signal transduction pathways. *EMBO J* 12:4221–4228.

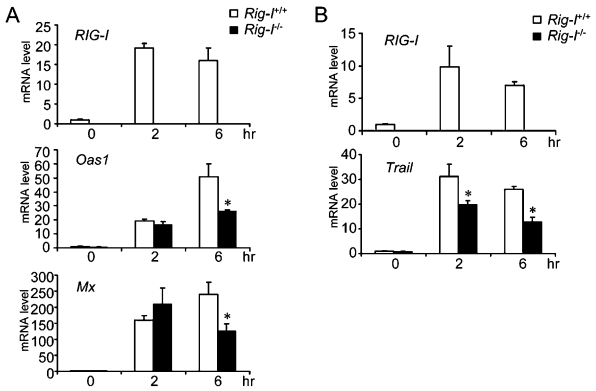

Fig. S4. ISG induction during the early phase is impaired by Rig-I deficiency. Primary *Rig-I*$^{+/+}$ or *Rig-I*$^{-/-}$ BM myeloid cells were stimulated by 1,000 U/mL IFN-α (*A*) or 30 ng/mL IFN-γ (*B*) for different time points as indicated. Relative mRNA levels of various ISGs were measured by real-time PCR assay (n = 3, mean ± SD; *$P < 0.05$).

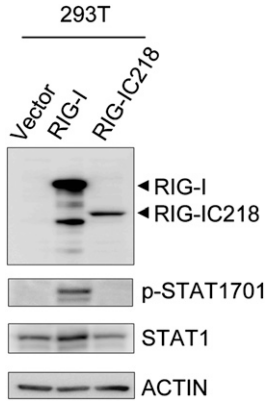

Fig. S5. STAT1 activation by RIG-I induction requires caspase recruitment domains (CARDs). RIG-IC218–expressing plasmid, which encodes a RIG-I mutant ranging from amino acids 218–925 of the WT RIG-I (with the deletion of the N-terminal CARDs), is transiently transfected into 293T, in parallel with the transfection of a full-length RIG-I–encoding plasmid. After 48 h, the tyrosine-phosphorylated and total levels of STAT1 were examined by Western blotting assay.

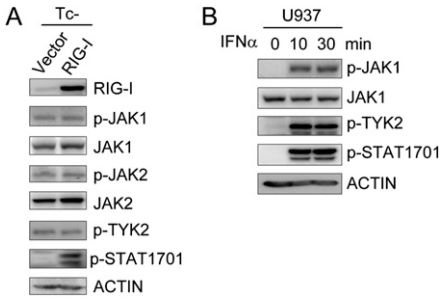

Fig. S6. RIG-I induction does not promote the activation of JAK family members. (*A*) U937/vector and U937/RIG-I cells were cultured in the Tc^{-} medium for 6 d, and the cell protein extracts were detected by Western blotting analysis for phosphorylated levels of JAK1, JAK2, and TYK2. (*B*) U937 cells were treated with 1,000 U/mL IFN-α for the indicated time points, and the cell lysates were analyzed by Western blotting assay.

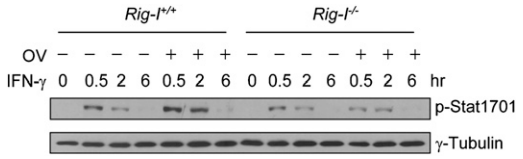

Fig. S7. Phosphatase inhibitor treatment fails to rescue the hypophosphorylated status of Stat1 in *Rig-I*$^{-/-}$ myeloid cells. *Rig-I*$^{+/+}$ and *Rig-I*$^{-/-}$ BM myeloid cells were treated with 30 ng/mL IFN-γ, with or without 0.1 mM sodium orthovanadate (OV), for different time points as indicated. Cells were then collected for protein extracts and detected by Western blotting analysis for tyrosine 701-phosphorylated Stat1.

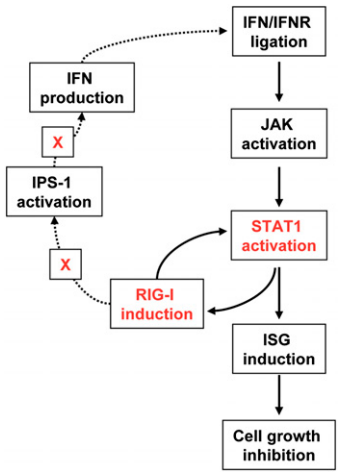

Fig. S8. Diagram depicts an amplifying role of RIG-I induction on STAT1 activation. Within the canonical IFN signaling pathway, IFN ligands bind to their receptors on the membrane and the activated JAK family kinases trigger the phosphorylation/activation of STAT1, which, in turn, induces the expression of numerous ISGs, including RIG-I. Our data indicate that RIG-I induction exerts a positive feedback effect on STAT1 activation, which critically amplifies ISG expression and cellular growth-inhibiting effect. In addition, RIG-I induction triggers STAT1 activation via a mechanism independent of further stimulating IPS-1 or JAKs, indicating the existence of a noncanonical STAT1 activation pathway through which RIG-I induction acts.

Table S1. siRNA sequences for the indicated genes

Knockdown constructs	Target sequences(5′-3′)
RIG-Ish1	TGCAATCTTGTCATCCTTTAT
RIG-Ish2	AAATTCATCAGAGATAGTCAA
IPS-1sh1	CCTGGTGCAGTGCCTTCTAAT
IPS-1sh2	GGCAGGTCAGTTAACAATTTA
STAT1sh1	GCAAGCGTAATCTTCAGGATA
STAT1sh2	CCTGAAGTATCTGTATCCAAA
NC	GCTCCCGTGAATTGGAATCCT

NC, negative control.

Table S2. Primers used for real-time PCR assays

Genes	Forward primers (5′-3′)	Reverse primers (5′-3′)
Human RIG-I	GGACGTGGCAAAACAAATCAG	GCAATGTCAATGCCTTCATCA
Human STAT1	ACCGCACCTTCAGTCTTTTCC	TGAACTGGACCCCTGTCTTCA
Human TRAIL	GAAGCAACACATTGTCTTCTCCAA	TGATGATTCCCAGGAGTTTATTTTG
Human KLF4	TCCCGCCGCTCCATTAC	CGATCGTCTTCCCCTCTTTG
Human OAS1	CTCATCCGCCTAGTCAAGCACT	CAAGCATAGACCGTCAGGAGCT
Human IFN-β	CAGCAATTTTCAGTGTCAGAAGCT	TCATCCTGTCCTTGAGGCAGTA
Human PKR	AGCAAAACAATTGGCCGC	AGCGAGTGTGCTGGTCACTAA
Mouse Rig-I	GGACGTGGCAAAACAAATCAG	GCAATGTCAATGCCTTCATCA
Mouse Icsbp	GCCATACAAAGTTTACCGAATTGTTC	TCACGCAGCCAGCAGTTG
Mouse Trail	GAAGCAACACATTGTCTTCTCCAA	TGATGATTCCCAGGAGTTTATTTTG
Mouse Klf4	TCCCGCCGCTCCATTAC	CGATCGTCTTCCCCTCTTTG
Mouse Oas1	GCCTGGTCACGCACTGGTA	AAGCCCTGGGCTGTGTTG
Mouse Pkr	GCTGCGAAAGAAGCCTATCAGA	TGCTGGAAAAGCCACTGAATG
Mouse Mx	TCCCAGACCTGACTCTCATTGA	GGTTGATGGTCTCTTGTTTTTGG
18s	CGCGGTTCTATTTTGTTGGTTT	TTCGCTCTGGTCCGTCTTG