Supplemental Information

DDX19 Inhibits Type I Interferon Production by Disrupting TBK1-IKKε-IRF3 Interactions and Promoting TBK1 and IKKε Degradation

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Figure S1. DDX19 inhibits type I IFN production. Related to Figure 1.

(A) Schematic of the structure of human DDX19A and DDX19B proteins.
(B) Alignment of 1–60 amino acid sequences of human DDX19A and DDX19B by Clustal Omega.
(C) The specificities of anti-DDX19A and DDX19B antibodies were analyzed by Western blotting.
(D and E) qPCR was performed to analyze the mRNA levels of Ddx19a (D) and Ddx19b (E) genes in different tissues of C57B6/J mice.
(F–I) HEK293T cells were transfected with an IFNβ reporter or an ISRE reporter and a Renilla-TK reporter, along with increasing amounts of a plasmid expressing DDX19B. At 24 hpt, the cells were transfected with poly(I:C) or infected with SeV. A luciferase assay was performed.
(J and K) THP-1 cells (J) and RAW 264.7 cells (K) were incubated with poly(I:C) or infected with SeV at the indicated times. The expression of DDX19 was detected by Western blotting.

**p < 0.01 and ***p < 0.001 (one-way ANOVA followed by Bonferroni post-test (F–I)). Data are from three independent experiments (F–I; mean ± SD of triplicate assays) or are representative of three independent experiments with similar results (C, J–K).
Figure S2. DDX19 suppresses the phosphorylation of IRF3, which is independent of ATPase or RNA binding activities. Related to Figure 1.

(A) Schematic of full-length DDX19 and its mutants.

(B and C) HEK293T cells were transfected with an IFNβ reporter (B) or an ISRE reporter (C) and a Renilla-TK reporter, along with increasing amounts of a plasmid expressing DDX19-M1, DDX19-M2, or DDX19-M3. At 24 hpt, the cells were infected with SeV. A luciferase assay was performed.

(D) HEK293T cells were transfected with a plasmid encoding HA-IRF3, along with increasing amounts of a plasmid encoding Flag-tagged DDX19-WT, DDX19-M1, DDX19-M2, or DDX19-M3, and then the cells were mock-infected or infected with SeV as indicated. The phosphorylation levels of IRF3 were analyzed by Western blotting.

***p < 0.001 (one-way ANOVA followed by Bonferroni post-test (B–C)). Data are from three independent experiments (B–C; mean ± SD of triplicate assays) or are representative of three independent experiments with similar results (D).
Figure S3. DDX19 inhibits RLRs-mediated IFNβ mRNA transcription. Related to Figure 2.
(A–F) HEK293T cells were transfected with a plasmid expressing Flag-tagged RIG-I (A), MDA5 (B), MAVS (C), TBK1 (D), IKKε (E), or IRF3-5D (F), along with increasing amounts of a plasmid encoding DDX19. At 24 hpt, the cells were harvested, and total RNAs were extracted. The mRNA levels of IFNβ were analyzed by qPCR. The results were normalized to β-actin mRNA. NS, not significant (p > 0.05); **p < 0.01 and ***p < 0.001 (one-way ANOVA followed by Bonferroni post-test). Data are from three independent experiments (A–F; mean ± SD of triplicate assays).
Figure S4. DDX19 interacts with IRF3. Related to Figure 3.

(A-B) HEK293T cells were transfected with a plasmid expressing HA-DDX19 or Flag-IRF3 alone or both plasmids as indicated (A) or transfected with a plasmid expressing Flag-DDX19 alone or along with a plasmid expressing HA-IRF3 (B). At 36 hpt, Co-IP was performed with anti-Flag.

(C-D) HEK293T cells (C) or RAW264.7 cells (D) were mock-infected or infected with SeV, and then, immunoprecipitation was performed with anti-DDX19 antibody. IgG was used as a negative control.

The whole-cell lysates and immunoprecipitants were analyzed by Western blotting.

(E) HeLa cells were transfected with a plasmid expressing HA-DDX19, Flag-IRF3 alone, or both plasmids. The cells were probed with mouse anti-HA polyclonal antibody and rabbit anti-Flag monoclonal antibody.

(F) The Zeiss processing system was used to generate a scatterplot of HA-DDX19 and Flag-IRF3 or...
DAPI from confocal images (B). Ch3-T1 denotes the 633 nm channel (Flag-IRF3), Ch2 GaAsP-T2 denotes the 488 nm channel (HA-DDX19), and Ch1-T3 denotes the 405 nm channel (DAPI).

(G) The Pearson’s correlation coefficient and Mander’s Overlap coefficient of images (B) were analyzed using the Zeiss processing system.

(H) The Zeiss processing system was used to generate a scatterplot of DDX19 and IRF3 or DAPI from (Fig. 3C) confocal images.

(I) The Pearson’s correlation coefficient and Mander’s Overlap coefficient of (Fig. 3C) images were analyzed using the Zeiss processing system.

(J) HEK293T cells were transfected with a plasmid expressing Flag-IRF3 along with a plasmid expressing HA-DDX19 or its truncated mutants, respectively. At 36 hpt, the cell lysates were immunoprecipitated with anti-Flag (M2) beads, and the whole-cell lysates and immunoprecipitants were analyzed by Western blotting.

(K) HEK293T cells were transfected with a plasmid expressing Flag-DDX19 along with a plasmid expressing HA-IRF3 or its truncated mutants. At 36 hpt, the cell lysates were immunoprecipitated with anti-Flag (M2) beads, and the whole-cell lysates and immunoprecipitants were analyzed by Western blotting.

Data are representative of three independent experiments with similar results (A–K).
Figure S5. Map the interaction domains of DDX19-TBK1, TBK1-IRF3, and IKKε-IRF3. Related to Figure 4.

(A and B) HEK293T cells were co-transfected with a plasmid expressing Flag-TBK1, and a plasmid expressing HA-DDX19 or its truncated mutants (A) or were transfected with Flag-DDX19 or its truncated mutants alone (B). At 36 hpt, the cell lysates were immunoprecipitated with anti-Flag (M2) beads, and the whole-cell lysates and Immunoprecipitants were analyzed by Western blotting.

(C and D) HEK293T cells were transfected with a plasmid expressing Flag-TBK1 (C) or Flag-IKKε (D) along with a plasmid expressing HA-IRF3 or its truncated mutants. At 36 hpt, the cell lysates were immunoprecipitated with anti-Flag (M2) beads, and the whole-cell lysates and Immunoprecipitants were then analyzed by Western blotting. Data are representative of three independent experiments with similar results (A–D).
Figure S6. Generation of Ddx19 knockout mice. Related to Figure 7.
(A) Schematic of the TALEN-mediated Ddx19 (Ddx19a and Ddx19b) gene knockout strategy in mice.
(B) PCR was used to identify the genotypes of offspring.
(C) The protein levels of DDX19 in MEFs and macrophages isolated from Ddx19+/+ or Ddx19b+/− mice were detected by Western blotting.
(D) The genotypes and phenotypes of the Ddx19-knockout mice were analyzed.
(E–F) Peritoneal macrophages (E) or the MEFs (F) isolated from Ddx19+/+ or Ddx19b+/− mice were infected with SeV at the indicated times and the cell lysates were analyzed by Western blotting.
Data are representative of three independent experiments with similar results (B–C, E–F).
Figure S7. DDX19 negatively regulates type I IFN production in vivo. Related to Figure 7.

(A–C) Schematic diagrams show that the CRISPR/Cas9-mediated gene knockout strategies for the Ddx19a, Ddx19b, and Ddx19 (Ddx19a and Ddx19b) in mice.

(D) The genotypes and phenotypes of Ddx19-knockout mice were analyzed.

(E–H) Ddx19<sup>+/−</sup> mice were generated using CRISPR/Cas9 technology to delete 4138 bp of the Ddx19b gene. Peritoneal macrophages isolated from Ddx19b wild type mice (Ddx19<sup>+/+</sup>) were stimulated with poly(I:C) for 12 h, and then, the levels of secreted IFNβ were analyzed by ELISA (E). The transcription levels of IFNβ (F), ISG54 (G), and ISG56 (H) in cells were evaluated by qPCR.

(I and J) Ddx19<sup>+/−</sup> mice were generated using CRISPR/Cas9 technology to delete 39002 bp of the Ddx19b and Ddx19a genes. Peritoneal macrophages isolated from Ddx19<sup>+/+</sup> and Ddx19<sup>+/−</sup> were stimulated with poly(I:C) (I) or infected with SeV (J) for 12 h, and then, the levels of secreted IFNβ were analyzed with ELISA.

*p < 0.05, **p < 0.01, and ***p < 0.001 (unpaired Student’s t-test (E–J)). Data are from three independent experiments (E–J; mean ± SD of triplicate assays).
**Table S1.** The TALEN pairs sequence used in this study. Related to STAR Methods.

| TALEN pair          | TALEN  | Target DNA sequence (5’-3’) | Spacer              |
|---------------------|--------|----------------------------|---------------------|
| *Ddx19b*-E6-59      | 59L    | TGGTACGGGTTAAACAGCTG       | CCTTTGGTTTTG       |
| (TALEN pair 1)      | 59R    | TGCAGGCTACTTTGCTGA         | TGTGC (18bp)       |
| *Ddx19a*-E12-292    | 292L   | TAGCCCTGGGTGTGGTGGT       | GGTGCACACTTTTGG    |
| (TALEN pair 2)      | 292R   | TCTACCCCTCAAAGTGTG        | TCC (18bp)         |

**Table S2.** The sgRNA pairs used in this study. Related to STAR Methods.

| sgRNA pair | sgRNA guide | Target sequence | sgRNA sequence (5’-3’) |
|------------|-------------|-----------------|------------------------|
| *Ddx19*   | 5’-7#-1     | CTGACAACGTGCAAGAATGG | TAGGACAACGTGCAAGAATGG |
|           | 3’-13#-1    | GGCTCAGAGTTAAAAGCAT | TAGGCTCAGAGTTAAAAGCAT |
| *Ddx19a*  | 4           | AGTGTTAGGTCTTCCACCT | TAGGTTAGGTCTTCCACCT |
|           | 13-1        | GGCTCAGAGTTAAAAGCAT | TAGGCTCAGAGTTAAAAGCAT |
| *Ddx19b*  | 6           | TGGCTGCTAAAGGAGACTG | TAGGCTGCTAAAGGAGACTG |
|           | 8           | TAGTCATACCATAAGGTC   | TAGTCATACCATAAGGTC   |
### Table S3. The primers used for mouse genotyping in this study. Related to STAR Methods.

| Genotype name                  | Primers   | Sequence (5’-3’)                                      | Product size |
|--------------------------------|-----------|------------------------------------------------------|--------------|
| TALEN-Ddx19-Δ30919             | Ddx19b-E2-F | CTGTCCACTGTGGCTGTCTAAAG                                | Mut: 880 bp  |
|                               | Ddx19a-E2-R | GGCCTGAGAGCCTATAACAGC                                  |              |
| Cas9-Ddx19-Δ39002             | Ddx19-GT-F  | GAAAGGTTCAACTCCTCC                                       | Mut: 650 bp  |
|                               | Ddx19-GT-R  | TCACAGTGAGCAACAAAT                                       |              |
| Cas9-Ddx19a-Δ19587            | Ddx19a-GT-F | AACTTAGGGATCTGGGGGA CAGACC                              | Mut: 700 bp  |
|                               | Ddx19a-GT-R | AGGTTCAACAGTGAGCACC                                       |              |
| Cas9-Ddx19b-Δ4138             | Ddx19b-GT-F | TCTCCCTTTCCAGATGCTA GTTGAG                              | Mut: 746 bp  |
|                               | Ddx19b-GT-R | CCAGCCTGGGCTATACGTA GATTCTA                             |              |
### Table S4. The primers used for qPCR in this study. Related to STAR Methods.

| Gene name   | Primers | Sequence (5’-3’) |
|-------------|---------|-----------------|
| Human IFN-β | hIFN-β- F | ATGACCAACAAGTGCTCCTCC |
|             | hIFN-β-R  | GCTCATGGAAAGAGCTGTAGTG |
| Human ISG54 | hISG54-F  | CTTCCCAGTCTATCATCAACCTT |
|             | hISG54-R  | CGTCGCTCTATGCTATCT |
| Human ISG56 | hISG56-F  | TCATCAGGTCAGGATAGTC |
|             | hISG56-R  | CCACACTGTATTTGTTGTCTAGG |
| Human TBK1  | hTBK1-F   | CGAAGCCGGAAGTGCCTCAG |
|             | hTBK1-R   | CTCTGCATCTTGGCTGGATCA |
| Human IKKε  | hIKKε-F   | CTCAGAGTGGGCGAGAAG |
|             | hIKKε-R   | TCCGCAAGGACCTCAACTC |
| Human β-actin | hβ-actin-F | CTTTCTGCGCATGGAGTCTC |
|             | hβ-actin-R | GGAGCAATGATCTGCTTCC |
| Mouse IFN-β | mIFN-β-F  | CCCTATGGGATGACGGAAGA |
|             | mIFN-β-R  | CTGTCTGGTGGGGGATCTCA |
| Mouse ISG54 | mISG54-F  | TCTCCAGTGACTCCTCTCC |
|             | mISG54-R  | CAGCAAGATGCAACAGATG |
| Mouse ISG56 | mISG56-F  | TCGATCCACAGTTGAACAC |
|             | mISG56-R  | ACTTCCGGGAATCGAGA |
| Mouse GAPDH | mGAPDH-F  | AAATGGGTAGGGTCGGTGTGAAC |
|             | mGAPDH-R  | CAACAATCTCCACTTGGCCACTG |

### Table S5. The sequence of siRNA used in this study. Related to STAR Methods.

| Gene Name   | sRNA Name | sRNA sequence (5’-3’) |
|-------------|-----------|----------------------|
| human Ddx19 | siDDX19-1# | UCAAGUCGAUGACCAUUUG |
|             | siDDX19-2# | UCAACACUGACUAGAAG |
| human Lamtor2 | si-Lamtor2 | GCCACCCUGCGUGGAAUUATT |