Supporting Online Material for

A novel lncRNA regulates HCV infection through IFI6

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Supplemental Materials and Methods

Cells and virus

Huh7.5.1, HepG2, LX2, TWNT4 and Huh7 cells were grown in DMEM (Thermo Fisher Scientific, Waltham, UK) supplemented with 10% fetal bovine serum (FBS) (GIBCO, city, state) and 1% penicillin/streptomycin (Invitrogen, city, state) at 37^{o}C with 5% CO_2. Huh7.5.1 cells were infected with genotype 2a JFH1 HCV virus at a multiplicity of infection of 0.2 (JFH1 cells) as previously described. Primary human hepatocytes (PHHs) were purchased from TRIANGLE Research Laboratories (Durham, NC), and cultured according to the manufacturer's instructions. In brief, PHHs were seeded onto matrix-coated plates and cultured with Hepatocyte Plating Medium (Durham, NC, USA). Cell viability was determined using a Cell Titer-Glo luminescent cell viability assay kit (Promega, Madison, WI) according to the manufacture`s protocol.

Long non-coding RNA high-throughput sequencing and data analysis

Total RNA from control Huh7.5.1 cells and IFNα-treated (30 IU/mL) Huh7.5.1 cells were used to prepare the lncRNA sequencing library. Oligo dT beads were used to select mRNA from the total RNA. The Illumina library preparation was performed by using broadly designed indexed adapters after cDNA was synthesized with RT primers. The samples were quantified by qPCR after enrichment, followed by lncRNA high-throughput sequencing performed at the Broad Institute (Cambridge, MA, USA) on the Illumina

HiSeq2000 platform using the Illumina Tru Seq™ Rapid SBS kit (#FC-402-4002, Illumina) according to the manufacturer's instructions. The significantly differentially expressed lncRNAs for the IFNα treated sample *vs.* control sample were determined by fold-change value (see Bioinformatic analysis below), where ≥ 2.0 fold-change differential gene expression indicates up-regulation of lncRNA expression in that the lncRNA in IFNα-treated samples, while a fold-change value ≤ 0.5 indicates down-regulation of lncRNA gene expression in IFNα treated samples. We then chose 5 up-regulated and 5 down-regulated IFN-induced lncRNAs from our RNAseq bioinformatic analysis for further validation.

RNA extraction and quantitative RT-PCR

Total cellular RNA from cells was harvested using the QIAshredder and RNeasy mini kits (QIAGEN, Valencia, CA) according to the manufacturer's instructions. RNA concentrations were determined using the NanoDrop ND-1000 spectrophotometer (NanoDrop Inc., DE, USA). cDNA was synthesized by reverse transcription using the High-Capacity cDNA Kit (Invitrogen, Carlsbad, CA). Quantitative PCR (qPCR) was performed using Power Up SYBR Green Master Mix (Thermo Fisher Scientific, MA, USA) using the QuantStudio3 platform (Thermo Fisher Scientific, MA, USA). The mRNA expression level of target genes was normalized to GAPDH by using the $2^{-\Delta\Delta Ct}$ method to obtain mRNA arbitrary units (fold-change). Primer sequences are listed in Supplemental Table (STab 2).

Bioinformatics Analysis

The sequences of lncRNAs were obtained from NCBI and UCSC Genome Bioinformatics (http://genome.ucsc.edu/index.html). Novel human-encoded lncRNAs were analyzed using ChIPBase v2.0 (http://rna.sysu.edu.cn/chipbase/). The NCBI ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and coding potential calculator (http://cpc.cbi.pku.edu.cn) were used to predict the protein coding potential of lncRNAs. The RNAfold web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) was used to predict the secondary structure of lncRNAs and mutated lncRNAs. The precursor mRNAs of ISGs were predicted by using the UCSC genome browser (http://genome.ucsc.edu/index.html) and the NCBI Genome database (http://www.ncbi.nlm.nih.gov/genome/). The promoters of ISGs were predicted using Softberry (http://www.softberry.com/). The UCSC genome browser database (http://genome.ucsc.edu/index.html) was used to predict CpG islands.

CRISPR/Cas9 guide RNA (gRNA) editing

In order to study the biological function of lncRNA-IFI6, we used 3 CRISPR/Cas9 guide RNAs (gRNAs) (pSpCas9 BB-2A-Puro PX459 all-in-one system, GenScript Corporation, NJ, USA) to knock out lncRNA-IFI6. We tested 3 lncRNA-IFI6 gRNAs: CRISPR/Cas9 gRNA lncRNA-IFI6 #1 (5'-GTGCGGATTCGCACGGTGTT-3') which targets lncRNA-IFI6 (91-110

basepairs, bp), gRNA lncRNA-IFI6 #2 (5'-GGATTCTGTGCGGATTCGCA-3') which targets lncRNA-IFI6 (185-166 bp), and gRNA lncRNA-IFI6 #3 (5'-CAGAAGCCTCTCGTACGGTG-3') which targets lncRNA-IFI6 (84-103 bp). We selected gRNA #1 for further study based on the knockout effect. We also used 3 CRISPR/Cas9 guide RNAs (gRNAs) (pSpCas9 BB-2A-Puro PX459 all-in-one system, GenScript Corporation, NJ, USA) to knock out IFI6, CRISPR/Cas9 gRNA IFI6 #1 (5'- GCAGCGAGTAAACGGTTCTC-3') which targets IFI6 (155-174 bp), CRISPR/Cas9 gRNA IFI6 #2 (5'-AGATCCTAACTGGAAGAGTT-3') which targets IFI6 (158-177 bp), CRISPR/Cas9 gRNA IFI6 #3 (5'- GCATGCTTGTAATCCTACTT-3') which targets IFI6 (151-170 bp).

Plasmid information and cell transfections

Full-length lncRNA-IFI6 and truncated mutants of lncRNA-IFI6 (mutants 1-4) were synthesized and cloned into a pcDNA3.1(+) vector, carrying a neomycin resistance gene (GenScript corporation). Truncated lncRNA-IFI6 mutant domains were determined based on their secondary structure. Mutant 1 included lncRNA-IFI6 1-282 bp and 471-489 bp (large left arm and central circle structure), mutant 2 contained lncRNA-IFI6 261-489 bp (large right arm and central circle structure), mutant 3 contained lncRNA-IFI6 16-181 bp (small left arm), and mutant 4 contained lncRNA-IFI6 1-15 bp, 182-282 bp and 471-489 bp (central circle structure). IFI6 and OAS3 Gluc-On promoter reporter clones (pEZX-PG04-IFI6 and pEZX-PG04-OAS3) carrying the Gaussia luciferase (Gluc) and Secreted Alkaline Phosphatase (SEAP) were obtained

from GeneCopoeia (San Diego, USA). IFI6 expression, OAS3 expression and pcDNA3.1(+) empty vectors were purchased from GenScript Corporation. The pISRE-luc (expressing firefly luciferase) and pRL-TK (expressing *renilla* luciferase) were obtained from Promega (Madison, WI). The plasmids were transfected into cells using Lipofectamine LTX&PLUS Reagent (Invitrogen, MA, USA.) according to the manufacturer's instructions. Total RNA or protein was harvested at 72 hrs post vector transfection, 48 hrs post JFH1 HCV infection and 24 hrs post IFNα treatment at 30 IU/mL (PBL assay science, MA, USA).

Western Blot

Cells were washed with PBS and lysed using protein RIPA Lysis Buffer containing protease inhibitor cocktail (Sigma Life Science and Biochemicals, St. Louis, MO). Equal quantities of protein (20 µg) were loaded into each lane. Protein samples were separated by SDS-PAGE gel electrophoresis with NuPAGE Novex pre-cast 4-12% Bis-Tris gradient gels (Invitrogen, Carlsbad, CA) and blotted onto nitrocellulose membranes. The membranes were blocked with 5% bovine serum albumin (BSA). Targeted proteins were detected with specific primary antibodies including rabbit polyclonal antibody to IFI6 (Abcam, Cambridge, USA), mouse anti-β-Actin (Sigma Life Science and Biochemicals, St. Louis, MO), goat anti-OAS3 (Santa Cruz Biotechnology, USA), mouse anti-STAT1 (Cell signaling, USA) and mouse anti-HCV core C7-50 (Fisher Scientific, Pittsburgh, PA). The secondary antibodies were

horseradish peroxidase (HRP)-conjugated ECL donkey anti-rabbit IgG and HRP-conjugated ECL sheep anti-mouse IgG (GE Healthcare Biosciences, Pittsburgh, PA). The blots were detected by chemiluminescence using the Amersham ECL Western blotting detection kit (GE Healthcare Biosciences, Pittsburgh, PA).

Chromatin Immunoprecipitation (ChIP) assay

plncRNA-IFI6-overexpression, lncRNA-IFI6 gRNA, or negative control transfected Huh7.5.1 cells were selected for ChIP assays using the EZ-Magna ChIP A/G chromatin immunoprecipitation kit (Millipore, USA) according to the manufacturer's instructions. Briefly, cells were fixed with 1% formaldehyde for 10 minutes followed by quenching of the formaldehyde by rinsing with 10x glycine. The cells were then lysed in lysis buffer. The nuclear fraction of cells was pelleted in nucleus lysis buffer. The chromatin was ultrasonically treated and immunoprecipitated with the following antibodies: anti-H3K4me3 (Cat# 17-614), anti-H3K27me3 (Cat# 07-449), anti-RNA Polymerase II (Cat# 05-623B) and IgG control (Cat# 12-371B) (Millipore, MA). Elution of protein/DNA complexes and reverse cross-links of protein/DNA complexes to free DNA were performed using the ChIP Elution Buffer mixed with proteinase K, and the Magna Chip magnetic device. The DNA was purified for qRT-PCR to assess the level of IFI6 promoter and GAPDH promoter.

Generation of lncRNA CRISPR/Cas9 knockout stable cell lines

The CRISPR wild-type SpCas9 (WT SpCas9) system and pSpCas9 BB-2A-Puro (PX459) all-in-one vector driven by CBh promoter (drives expression of gRNA and Cas9 in a 1:1 ratio) were purchased from GenScript Corporation. The gRNAs (lncRNA-IFI6 gRNA and Neg gRNA) were transfected into cells by using Lipofectamine LTX&PLUS^{TM} reagent, in accordance with the manufacturer's instructions. Puromycin (Cell culture gibco, MA, USA) was used to select CRISPR/Cas9 stably transfected cell lines.

Small interfering RNA (siRNA) and transfection

The Dharmacon ON-TARGET plus SMART pool Human siRNAs (Fisher Scientific Life Science Research, Pittsburgh, PA) used for gene knock down were as follows: IFI6, STAT1, STAT2, JAK1, and non-target negative control siRNA (Neg-siRNA). The siRNAs were transfected into cells by using Lipofectamine RNAiMAX reagent (Invitrogen, MA, USA.), in accordance with the manufacturer's instructions. At 24 hrs post siRNA transfection, the cells were infected with HCV (JFH1) at 0.2 multiplicity of infection (MOI). At 48 hrs post siRNA transfection, human IFNα was added at a final concentration of 30 (IU/mL). The total RNA or protein lysates were harvested at 72 hrs post siRNAs transfection.

ISRE-luciferase reporter assay.

IFN-induced ISRE signaling was monitored using a luciferase reporter assay system driven by ISRE. pISRE-luc (expressing firefly luciferase) and pRL-TK (expressing *renilla* luciferase) were co-transfected into lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6 and pEmpty stably expressing Huh 7.5.1 cells, respectively. The luciferase lysates were harvested at 72 hrs post vector transfection, 48 hrs post JFH1 HCV (JFH1 at 0.2 MOI) infection, and 24 hrs post IFNα treatment. IFNα-stimulated ISRE luciferase levels were normalized to RL-TK luciferase levels yielding the relative luciferase unit (RLU).

Subcellular fractionation

Cytoplasmic and nuclear fractions of the cells were separated by using the SurePre^{TM} Nuclear or Cytoplasmic RNA purification kit (Fisher scientific, Canada), in accordance with the manufacturer's instructions. In brief, Huh7.5.1 cells were treated with IFNα 30 IU/mL for 24 hours. After washing the cells with PBS, cells were lysed with ice-cold Lysis Solution, and the cell lysates were centrifuged at 13,000 RPM for 15 min at 4^{o}C, after which the cytoplasmic RNA was located in the supernatant, and the nuclear RNA located within the pellets. The cytoplasmic RNA fraction and nuclear RNA fraction were individually bound to a column using a binding solution and washed three times. The cytoplasmic RNA and nuclear RNA were eluted in RNA elution solution.

5-Aza-2'-deoxycytidine treatment

Cells overexpressing lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6, pEmpty were treated with or without 10 μM 5-Aza-2'-deoxycytidineDNA (DAC) (methyltransferase inhibitor decitabine) (Sigma, USA) for 48 hours. Total RNA was isolated using the Total RNA kit (Qiagen, MA, USA). The mRNA level of each sample was detected by qRT-PCR.

RNA stabilization assays

lncRNA-IFI6 gRNA or plncRNA-IFI6 stably transfected Huh7.5.1 cells were treated with actinomycin D (ActD, Sigma, USA) at 1 μg/mL, cells were harvested at 0, 5, 10 and 15 hours post-treatment. The mRNA level was detected by qRT-PCR.

The value of fraction of RNA remained at 0 hr is taken as 100%, and the values of 5 hrs, 10 hrs and 15 hrs time points are normalized to 0 hr to calculate the RNA degradation rate.

Secrete-Pair Dual Luminescence analysis (Detection of ISGs promoter activity)

The Secrete-Pair Dual Luminescence Assay Kit for parallel bioluminescence assays of Gaussia luciferase (Gluc) and secreted Alkaline Phosphatase (SEAP) were provided by GeneCopoeia Inc (USA). Cells overexpressing plncRNA-IFI6 and lncRNA-IFI6 gRNA were transfected with a pEZX-PG04-IFI6-promoter and pEZX-PG04-OAS3-promoter, respectively. Guassia

luciferase (Gluc) assay and secreted Alkaline Phosphatase (SEAP) assays were performed to detect the IFI6 and OAS3 promoter activity.

Supplemental Table 1. List of identified long non-coding RNA from RNA-Seq

Supplemental Table 1 can be obtained online by link:

https://drive.google.com/file/d/0B5t4LtawAMViZWRHRUVpZmVIcTA/view?usp=sharing

Supplemental Table 2. List of used primer sequences

Stable 2. List of used primer sequence

LncRNA name	Accession number	Forward primer	Reverse primer
RP11-288L9.4 (lncRNA-IFI6)	HG491811.1	GAATCCGTTTCCTCTCCGCA	CTTTCACGTGCCAAACACCG
AP001610.5	HG511840.2	ATCAGCCTTGGGGGAGTGAA	GAGCTGGAGAAGCACATGTCA
RP11-670E13.5	HG509260.1	TCAAACCTTCTAAGAGGAATCGG	GTGGGAGGTGGGATTTAGCC
RP1-71H24.1	HG505187.1	TCCTGGATGAACTCTCCCCG	TGGTTGTCTTCCTCAGTCCTC
RP11-395B7.7	HG500454.1	GAAAGCTCTGGAACACCCGA	GCTCAGGTCAGCAACCTCAT
RP1-168P16.2	HG508507.1	GTAAGGGCTCTGCGTGTCTT	CCAGGGTCCATGCAGTTCAA
RP11-717D12.1	HG495646.1	AAGGTTGGGGAAGTGCTGAG	AATAGAGGCATGCACGCAGT
RP11-502N13.2	HG504542.1	CACCACCCTGTCAGCTATGTT	AGCTGCTGAGTCCAGAACAA
RP11-366L20.3	HG504909.1	TGGAAAGCTTTTTGGACGCC	CGCCTGTATGGCTTTGCATC
RP11-326L2.1	HG500922.1	TCCCAGCACTTGGCAAAAGA	CACTGGCATACCAGAGGCAT

Gene name	Accession number	Forward primer	Reverse primer
Human IFI6	NM_022873.2	GGTCTGCGATCCTGAATGGG	TCACTATCGAGATACTTGTGGGT
Human OAS3	NM_006187.3	GCTTCAAGAGCTATGTGGACC	GGAAACGTGAGTCTCAGACCA
Human IFIT1	NM_001270930.1	TTGATGACGATGAAATGCCTGA	CAGGTCACCAGACTCCTCAC
Human IFIT5	NM_012420.2	GGCCAAAATAAAGACGCCCTT	GACCAGGCTTCGTACTTCTTC
Human MX1	NM_001144925.2	GTTTCCGAAGTGGACATCGCA	GAAGGGCAACTCCTGACAGT
Human ZAP	XM_011516412.2	CCGGTGCAACTATTCGCAGT	TCAGTCCAGAGAGTTCGTGATTT
Human IRF7	NM_004031.2	GCTGGACGTGACCATCATGTA	GGGCCGTATAGGAACGTGC
Human ISG15	NM_005101.3	TCCTGGTGAGGAATAACAAGGG	GTCAGCCAGAACAGGTCGTC
Human ISG20	NM_001303234.1	CTCGTTGCAGCCTCGTGAA	CGGGTTCTGTAATCGGTGATCTC
Human STAT1	NM_007315.3	GAGAGCTGTCTAGGTTAACGTTCGC	AGTCAAGCTGCTGAAGTTCGTACC
Human JAK1	NM_001321857.1	GTGCTCCGCGCGCTCTTGAC	CTTCTTTGGAGAATGACGCC
JFH1-HCV	AB047639.1	TCTGCGGAACCGGTGAGTA	TCAGGCAGTACCACAAGGC
Human GAPDH	BC026907.1	ACCTTCCCCATGGTGTCTGA	GCTCCTCCTGTTCGACAGTCA
Human U6	NR_004394.1	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
IFI6 promoter	NM_022873.2	GCTGGTGATCAGGCTTCACT	AGCTTGATGCCCACACTTCA
GAPDH promoter	BC026907.1	TACTAGCGGTTTTACGGGCG	TCGAACAGGAGGAGCAGAGAGCGA
pcDNA3.1(+)	EF550208.1	CTAGAGAACCCACTGCTTAC	TAGAAGGCACAGTCGAGG

LncRNA name	Accession number	Forward primer	Reverse primer
IFI6-Intron1 (pre-IFI6-1)	U22970.1	TGATCCCTGGCTTGTGAACC	GATGTCTGCCATGGAAACGC
IFI6-Intron2 (pre-IFI6-2)	U22970.1	GTCTGGAGGGCGAGGATCT	GAGGAACAGGAGCAGGTGAG
IFI6-Intron3 (pre-IFI6-3)	U22970.1	GTCGCCAGAGTCCCAGATTC	ACAAAGCGTAGTGGGGTTGT
IFI6-Intron4 (pre-IFI6-4)	U22970.1	TGACATCTGCGGACGTTACC	GAATCCGTTTCCTCTCCGCA

Supplemental Figure

Supplemental Figure 1. IFNα-induced lncRNAs expression. We selected genes identified by RNAseq for further characterization. Each selected gene mRNA levels were normalized to GAPDH mRNA yielding arbitrary units (fold-change). * P < 0.05, ** P <0.01, and # P <0.001 for comparison between without or with IFN, respectively.

(A) The Venn distribution diagram of IFNα-induced lncRNAs. We identified 7901 lncRNAs by RNAseq in Huh7.5.1 cells. There were 495 downregulated and 567 upregulated IFNα-induced lncRNAs.

(B-F) The selected 5 IFN upregulated lncRNAs. We confirmed by qRT-PCR 5 IFN-induced lncRNAs including **(B)** lncRNA RP11-288L9.4, **(C)** lncRNA AP001610.5, **(D)** lncRNA RP11-670E13.5, **(E)** lncRNA RP11-473C18.3, **(F)** lncRNA RP11-395B7.7.

(G-K) The selected 5 IFN downregulated lncRNAs. (G) lncRNA RP1-168P16.2, **(H)** lncRNA RP11-717D12.1, **(I)** lncRNA RP11-502N13.2, **(J)** lncRNA RP11-366L20.3, and **(K)** lncRNA RP11-326L2.1.

(L) Paradigm of the genomic location of lncRNA gene RP11-288L9.4 (yellow arrow) and the relationship with the coding gene IFI6 (blue arrow).

(M) – (R) IFNα-induced lncRNA RP11-288L9.4 expression in several tested cells including (**M)** Huh7.5.1, **(N)** HepG2, **(O)** LX2, **(P)** TWNT4, **(Q)** Huh7, and **(R)** in human primary hepatocytes (PHH).

Supplemental Figure 2. lncRNA-IFI6 gRNA or plncRNA-IFI6 overexpression did not affect cell viability. Huh7.5.1 cells line stably expressing specific lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6 and pEmpty were generated. Cells were then inoculated with JFH1 HCV for 48 hrs. At 24 hrs post infection, human IFNα was added at a final concentration of 30 IU/mL. Total RNA or protein lysates were harvested at 48 hrs post infection. Cell viability was monitored using the Cell Titer-Glo luminescent cell viability assay kit. Data are shown as means ± standard deviation of three replicates. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(A) Paradigm of the lncRNA-IFI6 gRNA targeting sites.

(B) lncRNA-IFI6 gRNA did not affect Huh7.5.1 cell viability. Relative cell viability in Neg gRNA treatment was normalized to 100%.

(C) Over-expression of plncRNA-IFI6 did not affect Huh7.5.1 cell viability. Relative cell viability in pEmpty vector treatment was normalized to 100%.

Supplemental Figure 3. lncRNA-IFI6 regulation on HCV infection through IFI6 expression. JFH1 HCV was inoculated to the cells for 48 hrs. At 24 hrs post infection, human IFNα was added to a final concentration of 30 IU/ mL. The total RNA or protein lysates were harvested at 48 hrs post infection.

(A) lncRNA-IFI6 gRNA reduced HCV infection level in JFH1-infected Huh7.5.1 cells at 0, 12, 24, 36, 48, 72 hours post-infection compared to Neg gRNA respectively.

(B) Overexpression pIncRNA-IFI6 increased lncRNA-IFI6 RNA level in Huh7.5.1 or JFH1 cells compared to pcDNA Empty with or without IFN respectively.

(C) Overexpression of pIncRNA-IFI6 increased HCV RNA levels in JFH1-infected Huh7.5.1 cells at 0, 12, 24, 36, 48, 72 hours post-infection compared to pEmpty respectively.

(D) Overexpression of pIncRNA-IFI6 rescued IFN-induced inhibition of HCV RNA in JFH1-infected Huh7.5.1 cells compared to pEmpty respectively.

(E) pIncRNA-IFI6 overexpression reduced IFN-induced IFI6 mRNA levels compared to pEmpty.

(F) Overexpression of lncRNA-IFI6 increased HCV core protein levels and reduced IFI6 protein levels in JFH1-infected Huh7.5.1 cells.

(G) lncRNA-IFI6 gRNA decreased lncRNA-IFI6 levels in PHH and JFH1-infected PHH cells compared to Neg gRNA with or without IFN respectively.

(H) pIncRNA-IFI6 overexpression increased lncRNA-IFI6 RNA levels in PHH or JFH1-infected PHH cells compared to pEmpty with or without IFN respectively.

(I) pIncRNA-IFI6 overexpression rescued IFN-induced inhibition of HCV RNA in JFH1-infected PHH cells compared to pEmpty.

(J) The plncRNA-IFI6 overexpression reduced IFN-induced IFI6 mRNA levels compared to pEmpty in PHH cells.

Supplemental Figure 4. lncRNA-IFI6 specifically regulates IFI6. Huh7.5.1 cells stably expressing lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6 and pEmpty were generated. Cells were then inoculated with JFH1 HCV for 48 hrs. At 24 hrs post infection, human IFNα was added to a final concentration of 30 IU/mL. Total RNA or protein lysates were harvested at 48 hrs post infection. IFI6 and the selected ISG mRNAs were assessed by qRT-PCR. Data are shown as means ± standard deviation of three replicates. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(A) lncRNA-IFI6 gRNA significantly increased IFI6 mRNA expression in JFH1 cells (48 hrs post infection) compared to Neg gRNA with or without IFNα, respectively.

(B)-(I) lncRNA-IFI6 gRNA did not affect several ISGs including **(B)** OAS3, **(C)** IFIT1, **(D)** MX1, **(E)** IFIT5, **(F)** ZAP, **(G)** IRF7, **(H)** ISG15, **(I)** ISG20.

(J) plncRNA-IFI6 overexpression reduced IFNα-induced IFI6 mRNA levels compared to pEmpty.

(K)-(R) plncRNA-IFI6 overexpression did not affect several ISGs including **(K)** OAS3, **(L)** IFIT1, **(M)** MX1, **(N)** IFIT5, **(O)** ZAP, **(P)** IRF7, **(Q)** ISG15, **(R)** ISG20.

Supplemental Figure 5. lncRNA-IFI6 did not affect OAS3 mRNA and protein expression. Huh7.5.1 cells stably expressing specific lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6 and pEmpty were generated. Cells were inoculated with JFH1 HCV for 48 hrs. At 24 hrs post infection, human IFNα was added to a final concentration of 30 IU/mL. Total RNA or protein lysates were harvested at 48 hrs post infection. OAS3 mRNA was assessed by qRT-PCR. OAS3 and β-actin protein levels were detected by Western blot.

(A) lncRNA-IFI6 gRNA did not affect mRNA expression levels of OAS3.

(B) lncRNA-IFI6 gRNA did not affect OAS3 protein levels.

(C) plncRNA-IFI6 overexpression did not affect OAS3 mRNA expression levels.

(D) plncRNA-IFI6 overexpression did not affect OAS3 protein levels.

Supplemental Figure 6. IFI6 siRNA or pIFI6, lncRNA-IFI6 gRNA and plncRNA-IFI6 overexpression did not affect cell viability. Huh7.5.1 cells stably expressing lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6 and pEmpty were generated. IFI6 siRNA, Neg siRNA, pIFI6 and pEmpty were transfected into these Huh7.5.1 cells. Total RNA was harvested at 72 hr post vector transfection, 48 hr post JFH1 HCV infection (JFH1 at 0.2MOI), and 24 hr post IFNα treatment. Cell viability was monitored using the Cell Titer-Glo luminescent cell viability assay kit. Relative cell viability in Neg siRNA or pEmpty treatments was normalized to 100% respectively. Data are shown as

the mean ± standard deviation of three replicates. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(A) lFI6 siRNA did not affect JFH1 cell viability.

(B) Overexpression of plFI6 did not affect JFH1 cell viability.

(C) lFI6 siRNA significantly increased HCV RNA levels in JFH1 infected Huh7.5.1 cells compared to Neg siRNA with or without IFN respectively.

(D) lFI6 siRNA reduced lFI6 mRNA levels compared to Neg siRNA.

(E) lFI6 siRNA increased HCV core protein levels and reduced lFI6 protein expression compared to Neg siRNA.

(F) lFI6 siRNA in lncRNA-lFI6 gRNA stably transfected cells did not affect cell viability.

(G) Overexpression of plFI6 in cells overexpressing plncRNA-lFI6 did not affect cell viability.

(H) lFI6 siRNA rescued the reduction in HCV RNA mediated by lncRNA-lFI6 gRNA in JFH1 infected Huh7.5.1 cells compared to Neg siRNA.

(I) lFI6 siRNA blocked lFI6 mRNA enhancement in lncRNA-lFI6 gRNA treated JFH1 infected Huh7.5.1 cells compared to Neg siRNA.

(J) lFI6 siRNA rescued HCV core protein reduction and reversed lFI6 protein enhancement mediated by lncRNA-lFI6 gRNA in JFH1 infected Huh7.5.1 cells compared to Neg siRNA.

(K) Overexpression of pIFI6 rescued pIncRNA-IFI6 inhibition of IFI6 mRNA expression in JFH1 cells compared to pEmpty.

(L) IFI6 siRNA rescued HCV RNA reduction mediated by lncRNA-IFI6 gRNA in JFH1-infected PHH cells compared to Neg siRNA.

(M) IFI6 siRNA blocked IFI6 mRNA enhancement in lncRNA-IFI6 gRNA treated JFH1 infected PHH cells compared to Neg siRNA.

(N) Overexpression of pIFI6 inhibited pIncRNA-IFI6 induction of HCV RNA compared to pEmpty in JFH1-infected PHH cells.

(O) Overexpression of pIFI6 rescued pIncRNA-IFI6 inhibition of IFI6 mRNA expression in JFH1-infected PHH cells compared to pEmpty.

(P) Knock down of IFI6 did not affect lncRNA-IFI6 RNA expression.

(Q) Overexpression of pIFI6 did not affect lncRNA-IFI6 RNA levels.

Supplemental Figure 7. Cell viability of siRNAs to STAT1, STAT2, JAK1. The siRNAs to STAT1, STAT2, JAK1 were transfected into Huh7.5.1 cell for 24 hrs. IFNα (30 IU/mL) was added to the appropriate wells for 24 hrs. Cell viability was monitored using the Cell Titer-Glo luminescent cell viability assay kit. Relative cell viability in Neg siRNA or pEmpty treatment was normalized to 100% respectively. Data are shown as the mean ± standard deviation of three replicates. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(A) lncRNA-IFI6 gRNA did not significantly affect STAT1 mRNA levels compared to Neg gRNA in Huh7.5.1 or JFH1 cells with or without IFN respectively.

(B) lncRNA-IFI6 gRNA did not significantly affect STAT1 protein expression compared to Neg gRNA in Huh7.5.1 or JFH1 cells with or without IFN respectively.

(C) Overexpression of pIncRNA-IFI6 did not significantly affect STAT1 mRNA expression compared to pEmpty in Huh7.5.1 or JFH1 cells with or without IFN respectively.

(D) Overexpression of pIncRNA-IFI6 did not significantly affect STAT1 protein levels compared to pEmpty in Huh7.5.1 or JFH1 cells with or without IFN respectively.

(E) The siRNAs to STAT1, STAT2 or JAK1 did not significantly affect lncRNA-IFI6 mRNA levels in Huh7.5.1 cells with or without IFN respectively.

(F) JAK1 siRNA reduced JAK1 mRNA expression compared to Neg siRNA in Huh7.5.1 cells.

(G) STAT1 siRNA reduced STAT1 mRNA expression compared to Neg siRNA in Huh7.5.1 cells.

(H) STAT2 siRNA reduced STAT2 mRNA expression compared to Neg siRNA in Huh7.5.1 cells.

(I) siRNA targeting STAT1, STAT2, JAK1 did not affect Huh7.5.1 cell viability.

Supplemental Figure 8. Cell viability for supernatant incubation. pISRE-luc (expressing firefly luciferase) and pRL-TK (expressing *renilla* luciferase) were co-transfected in to lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6 and pEmpty stably expressing Huh 7.5.1 cells, respectively. IFNα-stimulated ISRE luciferase levels were normalized to RL-TK luciferase levels yielding the relative luciferase unit (RLU).

Huh7.5.1 cells stably expressing specific lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6 and pEmpty were generated, respectively. JFH1 HCV was inoculated to the cells for 48 hrs. At 24 hrs post infection, human IFNα was added to a final concentration of 30 IU/ mL. The supernatants of cells were harvested at 48 hrs post infection. The supernatants of these cells were used to incubate the fresh Huh7.5.1 cells.

(A) lncRNA-IFI6 gRNA did not significantly affect IFNα stimulated ISRE signaling compared to Neg gRNA in Huh7.5.1 and JFH1 cells.

(B) Overexpression of plncRNA-IFI6 did not significantly affect IFNα stimulated ISRE signaling compared to pEmpty in Huh7.5.1 and JFH1 cells.

(C) lncRNA-IFI6 gRNA or the ISRE-luciferase reporter assay did not affect Huh7.5.1 cell viability.

(D) pIncRNA-IFI6 overexpression or the ISRE-luciferase reporter assay did not affect Huh7.5.1 cell viability.

(E) Experimental schematic of supernatant incubation assay. To test whether lncRNA-IFI6 affects the total cytokine levels secreted by cells, fresh untreated Huh7.5.1 cells were incubated with the supernatant collected from Huh7.5.1 cells expressing lncRNA-IFI6 gRNA, Neg gRNA, pIncRNA-IFI6 or pEmpty for 24 hrs. Cell viability was monitored using the Cell Titer-Glo luminescent cell viability assay kit. Relative cell viability in Neg siRNA or pEmpty treatment was normalized to 100% respectively.

(F) Incubation of supernatants from lncRNA-IFI6 gRNA did not significantly affect IFI6 mRNA expression compared to Neg gRNA in Huh7.5.1 or JFH1 with or without IFN respectively.

(G) Incubation of supernatants from transfected cells overexpressing pIncRNA-IFI6 did not significantly affect IFI6 mRNA expression compared to pEmpty in Huh7.5.1 or JFH1 with or without IFN respectively.

(H) Supernatants from lncRNA-IFI6 gRNA transfected cells did not affect cell viability.

(I) Supernatants from transfected cells overexpressing pIncRNA-IFI6 did not affect cell viability.

Supplemental Figure 9. lncRNA-IFI6 regulates the initial transcription of IFI6. Cells stably over expressing lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6 and pEmpty were treated with or without 10 μM 5-Aza-2'-deoxycytidineDNA (DAC) (methyltransferase inhibitor decitabine) (Sigma, USA) for 48 hours. Relative cell viability in Neg gRNA or pEmpty treatment was normalized to 100% respectively.

(A) Paradigms of IFI6 pre-mRNA structures. IFI6 has 5 exons (Blue boxes) and 4 introns (Black lines). The CpG island (Green box) was located between exon 2 and intron 2 (4242-4545 bp) of the IFI6 gene.

(B) lncRNA-IFI6 gRNA and DAC treatment did not affect Huh7.5.1 cell viability.

(C) Overexpression of plncRNA-IFI6 and DAC treatment did not affect Huh7.5.1 cell viability.

(D) lncRNA-IFI6 gRNA significantly increased IFI6 pre-mRNA (pre-IFI6-2) levels compared to Neg gRNA in Huh7.5.1 or JFH1 cells with or without IFN respectively.

(E) Overexpression of plncRNA-IFI6 significantly reduced IFI6 pre-mRNA (pre-IFI6-2) levels compared to pEmpty in Huh7.5.1 or JFH1 cells with or without IFN respectively.

(F) ActD treatment inhibited IFI6 pre-mRNA expression levels in lncRNA-IFI6 gRNA stably transfected Huh7.5.1 cells. The IFI6 pre-mRNA values

were normalized to GAPDH.

(G) lncRNA-IFI6 gRNA did not significantly affect IFI6 pre-mRNA half-life compared to Neg gRNA in Huh7.5.1 cells. The pre-mRNA level at 0 hrs in each treatment in SFig 9F was set to 100% of the fraction value. The relative fraction values of each treatment at 5 hrs, 10 hrs or 15 hrs was obtained by normalizing to the value at 0 hr for each time point. The IFI6 pre-mRNA degradation half-life of Neg gRNA+ ActD is relatively equal to lncRNA-IFI6 gRNA+ ActD.

(H) ActD treatment reduced IFI6 pre-mRNA expression levels in plncRNA-IFI6 stably transfected Huh7.5.1 cells. The values were normalized to GAPDH.

(I) Overexpression of plncRNA-IFI6 did not significantly affect IFI6 pre-mRNA half-life compared to pEmpty in Huh7.5.1 cells. The pre-mRNA level at 0 hrs in each treatment in SFig 9H was set to 100% of the fraction value. The relative fraction values of each treatment at 5 hrs, 10 hrs or 15 hrs was obtained by normalizing to the value at 0 hr for each time point. The IFI6 pre-mRNA degradation half-life of plncRNA-IFI6 + ActD is relatively equal to pEmpty+ ActD.

Supplemental Figure 10. lncRNA-IFI6 regulates IFI6 through its promoter. Cells overexpressing lncRNA-IFI6 gRNA, Neg gRNA, plncRNA-IFI6 or pEmpty were co-transfected with pEZX-PG04-IFI6-promoter or pEZX-PG04-OAS3-promoter, respectively. Guassia luciferase (Gluc) assay and secreted Alkaline Phosphatase (SEAP) assay were performed to detect promoter

activity. Relative cell viability in Neg gRNA or pEmpty treatments was normalized to 100% respectively.

plncRNA-IFI6 overexpression, pEmpty, lncRNA-IFI6 gRNA, or Neg gRNA transfected Huh7.5.1 cells were subjected to ChIP. The relative amounts of IFI6 DNA immunoprecipitated by the H3K4me3 or H3K27me3 antibody were normalized to IFI6 DNA isolated by the control lgG. Data are shown as means ± standard deviation of three replicates. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(A) lncRNA-IFI6 gRNA did not affect pEZX-PG04-OAS3-promoter induced Gaussia luciferase (Gluc) / alkaline phosphatase (SEAP) activity compared to Neg gRNA in Huh7.5.1 or JFH1 cells with or without IFNα treatment.

(B) Overexpression of plncRNA-IFI6 did not affect pEZX-PG04-OAS3-promoter induced Gaussia luciferase (Gluc) / alkaline phosphatase (SEAP) activity compared to pEmpty in Huh7.5.1 or JFH1 cells with or without IFNα treatment.

(C) lncRNA-IFI6 gRNA and the IFI6 promoter reporter did not affect Huh7.5.1 cell viability.

(D) Overexpression of plncRNA-IFI6 and the IFI6 promoter reporter did not affect Huh7.5.1 cell viability.

(E) lncRNA-IFI6 gRNA and the OAS3 promoter reporter did not affect Huh7.5.1 cell viability.

(F) Overexpression of plncRNA-IFI6 and the OAS3 promoter reporter did not affect Huh7.5.1 cell viability.

(G)-(H) ChIP analysis of H3K4me3 (G) and H3K27me3 (H) levels at the IFI6 locus in lncRNA-IFI6 overexpressing and control cells. The relative amounts of IFI6 DNA immunoprecipitated by the H3K4me3 or H3K27me3 antibody were normalized to IFI6 DNA isolated by the control IgG.

(I) ChIP analysis of H3K4me3 levels at the GAPDH locus in lncRNA-IFI6 overexpressing and control cells.

(J) ChIP analysis of H3K27me3 levels at the GAPDH locus in lncRNA-IFI6 overexpressing and control cells.

(K) lncRNA IFI6 gRNA or overexpression of plncRNA-IFI6 did not affect Huh7.5.1 cell viability.

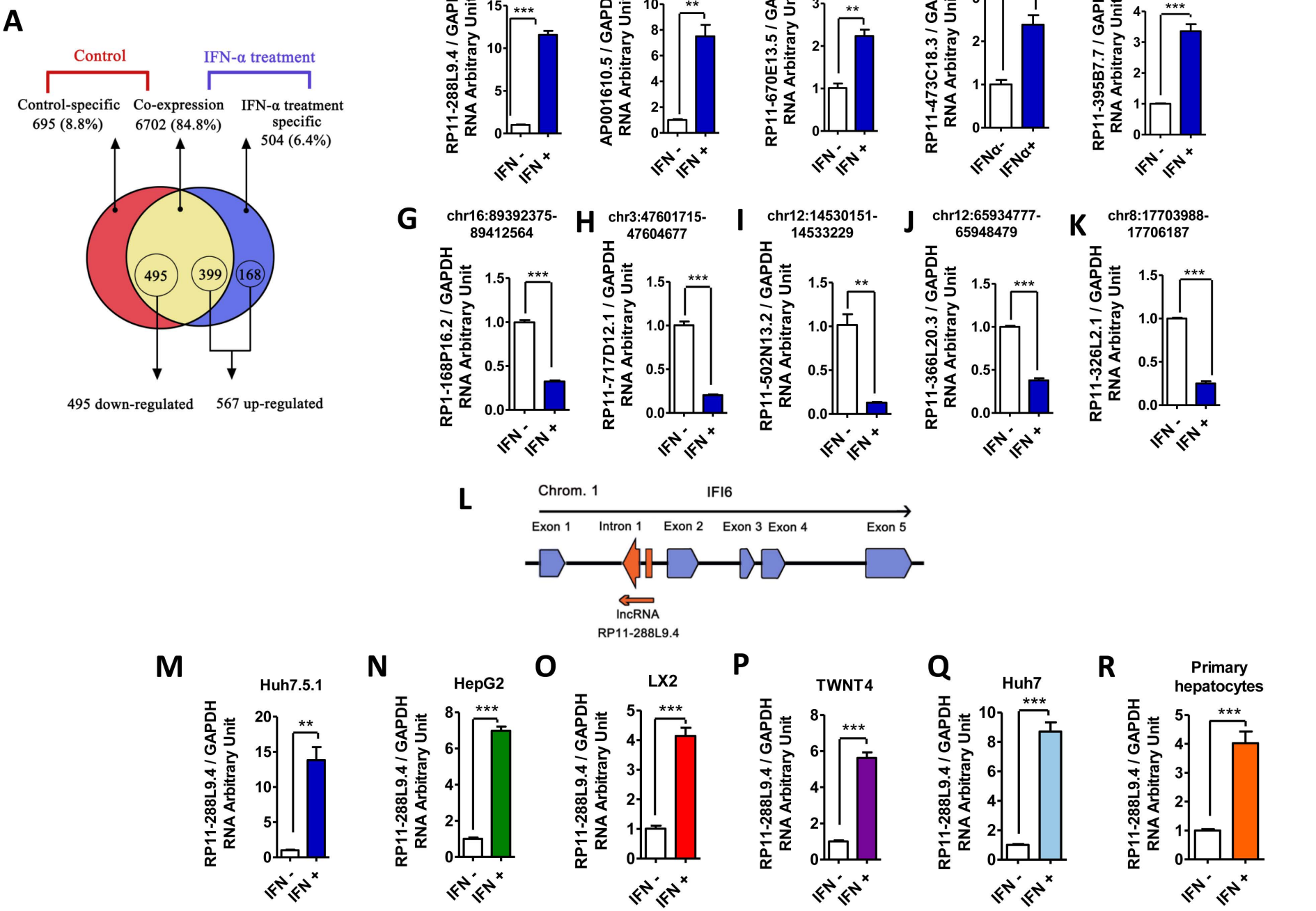
Supplemental Figure 11. Overexpression of wild type or mutant lncRNA-IFI6 did not affect cell viability. Huh7.5.1 cells stably expressing full-length plncRNA-IFI6, pMutants 1-4 and pEmpty were generated. Cells were inoculated with JFH1 HCV for 48 hrs. At 24 hrs post infection, human IFNα was added at final concentration of 30 IU/mL.

(A) Cell viability was monitored by Cell Titer-Glo luminescent cell viability assay kit. Relative cell viability in pEmpty treatment was normalized to 100% respectively.

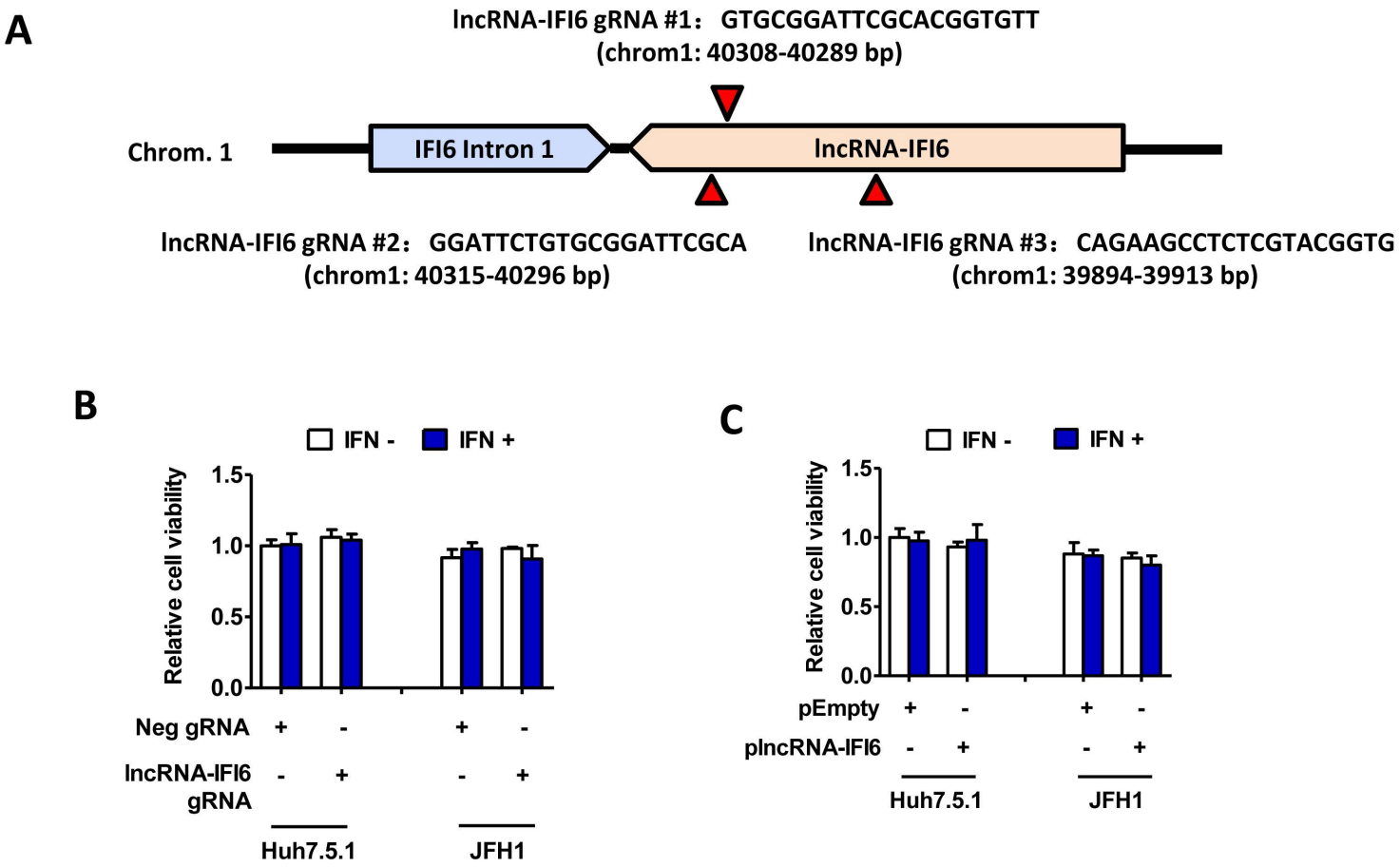
(B) Overexpression wild type or mutant 2 lncRNA-IFI6 significantly increased HCV RNA levels compared to pEmpty in JFH1 infected PHH cells with or without IFN respectively.

(C) Overexpression wild type or mutant 2 lncRNA-IFI6 significantly reduced IFI6 RNA level compared to pEmpty in JFH1 infected PHH cells with or without IFN respectively.

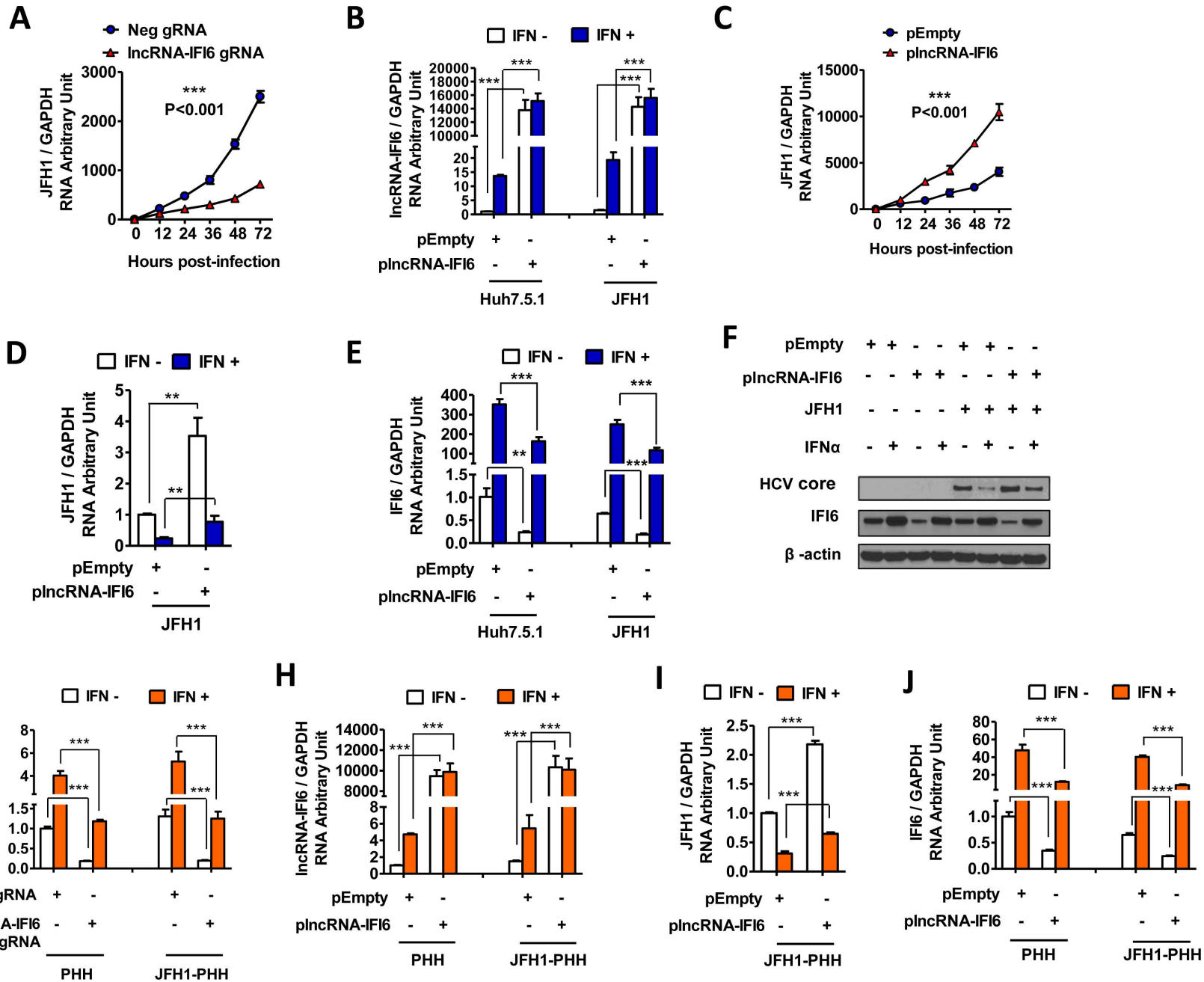
SFig 1.


A
Control
IFN-α treatment
Control-specific
695 (8.8%)
Co-expression
6702 (84.8%)
IFN-α treatment specific
504 (6.4%)
495
399
168
495 down-regulated
567 up-regulated
B
chr1:27669468-27670276
RP11-288L9.4 / GAPDH RNA Arbitrary Unit
C
chr21:41441056-41445708
AP001610.5 / GAPDH RNA Arbitrary Unit
D
chr17:56888880-56891841
RP11-670E13.5 / GAPDH RNA Arbitrary Unit
E
chr15:43184079-43185141
RP11-473C18.3 / GAPDH RNA Arbitrary Unit
IFNα-
IFNα+
F
chr7:101094047-101097967
RP11-395B7.7 / GAPDH RNA Arbitrary Unit
G
chr16:89392375-89412564
RP1-168P16.2 / GAPDH RNA Arbitrary Unit
H
chr3:47601715-47604677
RP11-717D12.1 / GAPDH RNA Arbitrary Unit
I
chr12:14530151-14533229
RP11-502N13.2 / GAPDH RNA Arbitrary Unit
J
chr12:65934777-65948479
RP11-366L20.3 / GAPDH RNA Arbitrary Unit
K
chr8:17703988-17706187
RP11-326L2.1 / GAPDH RNA Arbitrary Unit
IFN -
IFN +
L
Chrom. 1
IFI6
Exon 1
Intron 1
Exon 2
Exon 3
Exon 4
Exon 5
lncRNA
RP11-288L9.4
M
Huh7.5.1
N
HepG2
O
LX2
P
TWNT4
Q
Huh7
R
Primary hepatocytes
RP11-288L9.4 / GAPDH RNA Arbitrary Unit

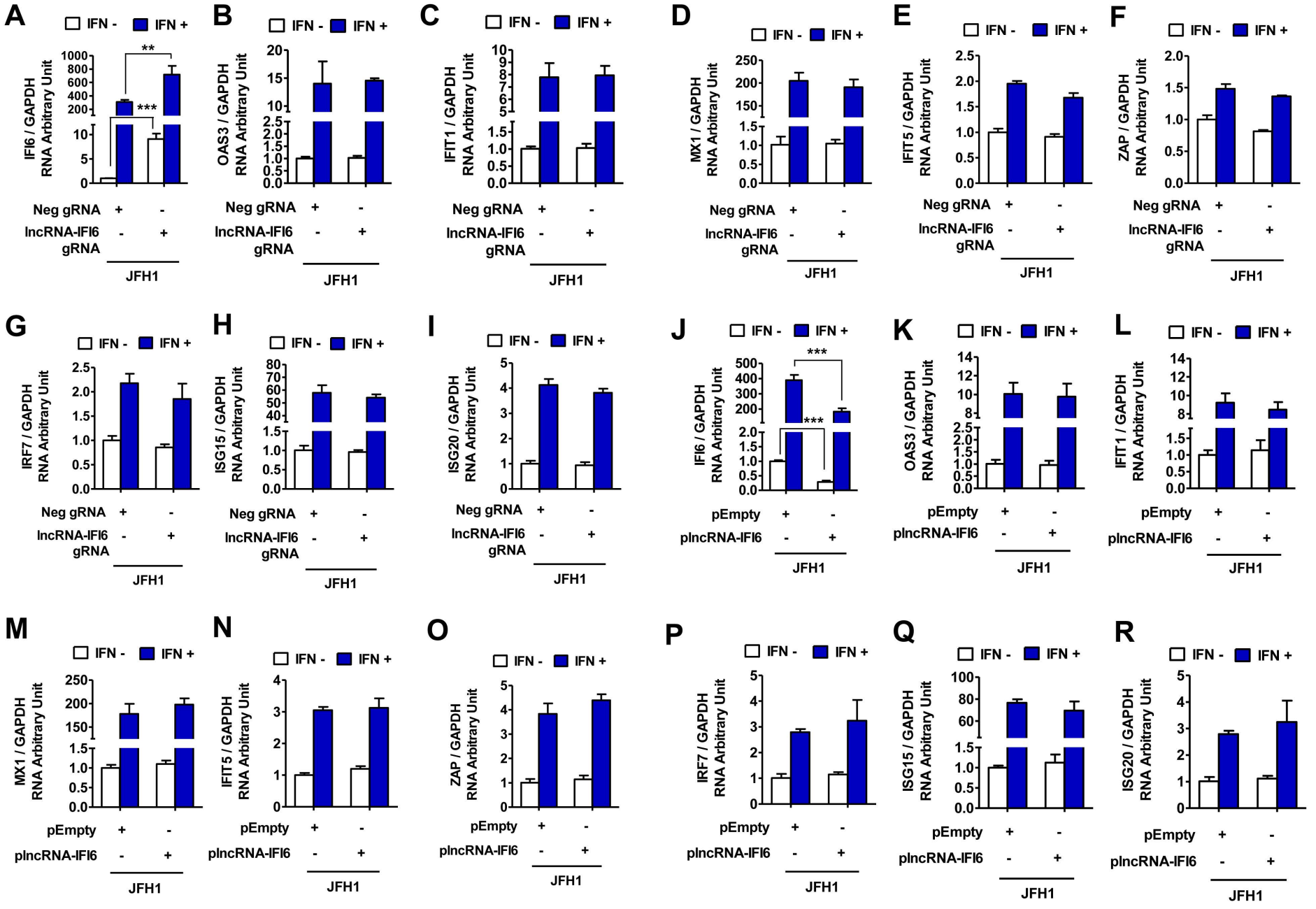
SFig 2.


A
lncRNA-IFI6 gRNA #1： GTGCGGATTCGCACGGTGTT
(chrom1: 40308-40289 bp)
Chrom. 1
IFI6 Intron 1
lncRNA-IFI6
lncRNA-IFI6 gRNA #2： GGATTCTGTGCGGATTCGCA
(chrom1: 40315-40296 bp)
lncRNA-IFI6 gRNA #3： CAGAAGCCTCTCGTACGGTG
(chrom1: 39894-39913 bp)
B
IFN -
IFN +
Relative cell viability
1.5
1.0
0.5
0.0
Neg gRNA + - + -
lncRNA-IFI6 gRNA - + - +
Huh7.5.1
JFH1
C
IFN -
IFN +
Relative cell viability
1.5
1.0
0.5
0.0
pEmpty + - + -
plncRNA-IFI6 - + - +
Huh7.5.1
JFH1

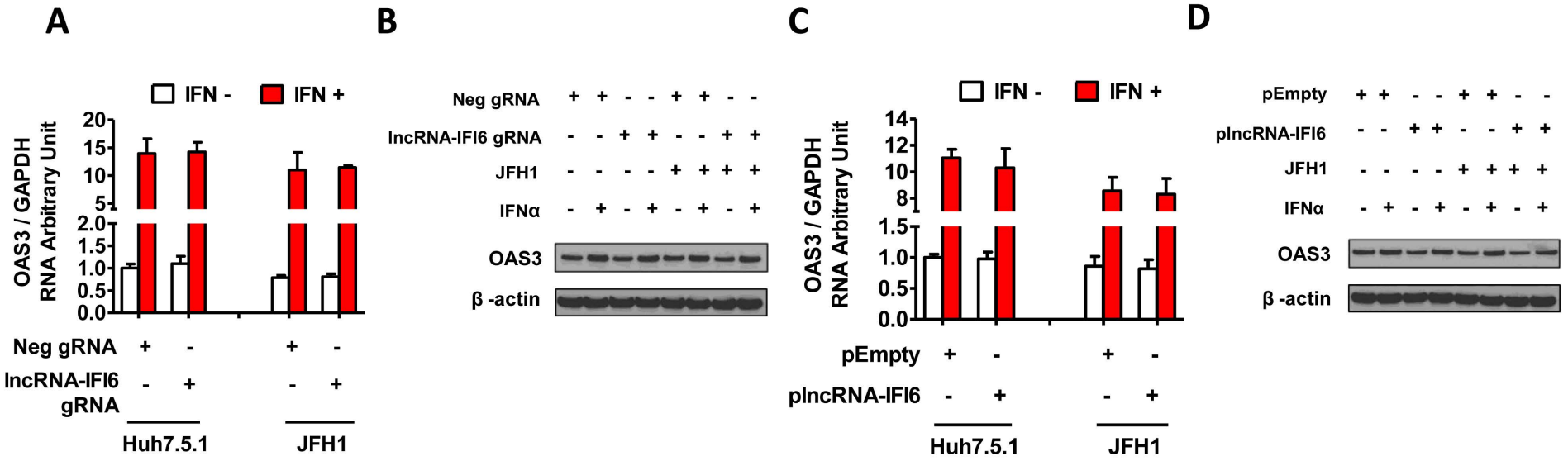
SFig 3.


A
Neg gRNA
lncRNA-IFI6 gRNA
JFH1 / GAPDH RNA Arbitrary Unit
***
P<0.001
Hours post-infection
B
IFN -
IFN +
lncRNA-IFI6 / GAPDH RNA Arbitrary Unit
pEmpty
pIncRNA-IFI6
Huh7.5.1
JFH1
C
pEmpty
pIncRNA-IFI6
JFH1 / GAPDH RNA Arbitrary Unit
***
P<0.001
Hours post-infection
D
IFN -
IFN +
JFH1 / GAPDH RNA Arbitrary Unit
pEmpty
pIncRNA-IFI6
JFH1
E
IFN -
IFN +
IFI6 / GAPDH RNA Arbitrary Unit
pEmpty
pIncRNA-IFI6
Huh7.5.1
JFH1
F
pEmpty
pIncRNA-IFI6
JFH1
IFNα
HCV core
IFI6
β -actin
G
IFN -
IFN +
lncRNA-IFI6 / GAPDH RNA Arbitrary Unit
Neg gRNA
lncRNA-IFI6 gRNA
PHH
JFH1-PHH
H
IFN -
IFN +
lncRNA-IFI6 / GAPDH RNA Arbitrary Unit
pEmpty
pIncRNA-IFI6
PHH
JFH1-PHH
I
IFN -
IFN +
JFH1 / GAPDH RNA Arbitrary Unit
pEmpty
pIncRNA-IFI6
JFH1-PHH
J
IFN -
IFN +
IFI6 / GAPDH RNA Arbitrary Unit
pEmpty
pIncRNA-IFI6
PHH
JFH1-PHH

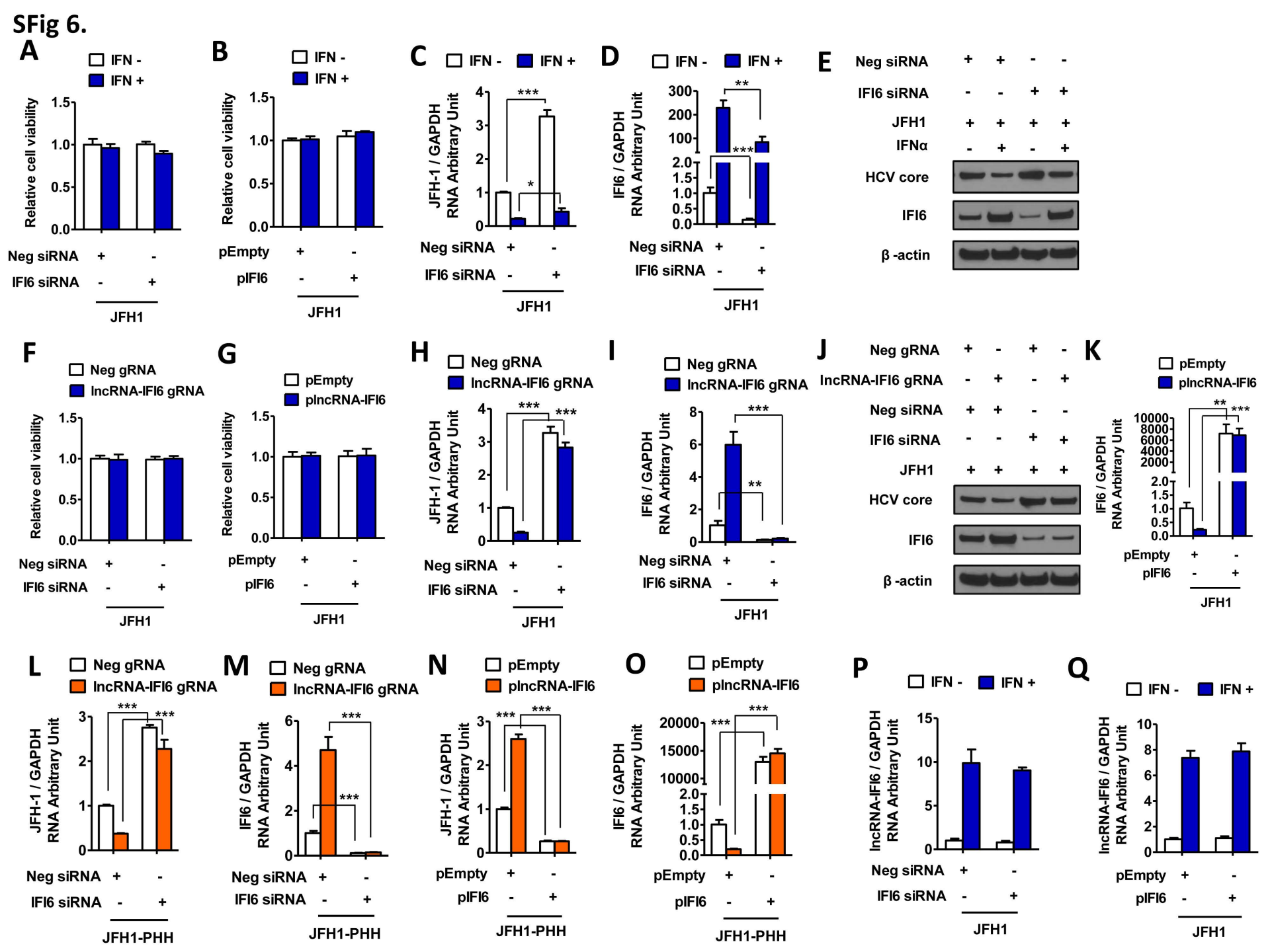
SFig 4.


A
IFN -
IFN +
IFI6 / GAPDH RNA Arbitrary Unit
**
***
Neg gRNA
lncRNA-IFI6 gRNA
JFH1
B
OAS3 / GAPDH RNA Arbitrary Unit
C
IFIT1 / GAPDH RNA Arbitrary Unit
D
MX1 / GAPDH RNA Arbitrary Unit
E
IFIT5 / GAPDH RNA Arbitrary Unit
F
ZAP / GAPDH RNA Arbitrary Unit
G
IRF7 / GAPDH RNA Arbitrary Unit
H
ISG15 / GAPDH RNA Arbitrary Unit
I
ISG20 / GAPDH RNA Arbitrary Unit
J
pEmpty
plncRNA-IFI6
K
L
M
N
O
P
Q
R

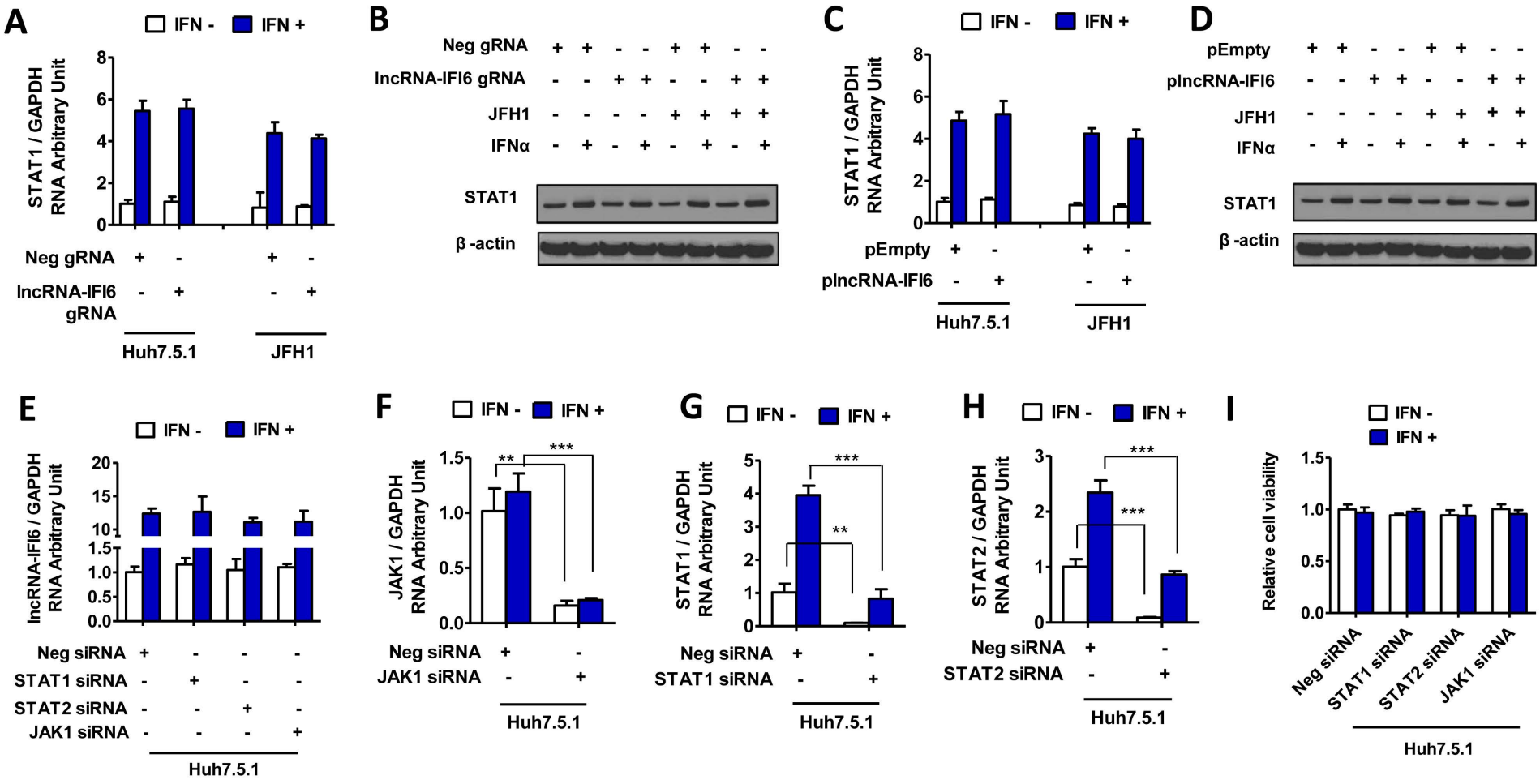
SFig 5.


A
IFN -
IFN +
OAS3 / GAPDH
RNA Arbitrary Unit
20
15
10
5
2.0
1.5
1.0
0.5
0.0
Neg gRNA + - + -
lncRNA-IFI6 gRNA - + - +
Huh7.5.1
JFH1
B
Neg gRNA + + - - + + - -
lncRNA-IFI6 gRNA - - + + - - + +
JFH1 - - - - + + + +
IFNα - + - + - + - +
OAS3
β -actin
C
IFN -
IFN +
OAS3 / GAPDH
RNA Arbitrary Unit
14
12
10
8
1.5
1.0
0.5
0.0
pEmpty + - + -
pIncRNA-IFI6 - + - +
Huh7.5.1
JFH1
D
pEmpty + + - - + + - -
pIncRNA-IFI6 - - + + - - + +
JFH1 - - - - + + + +
IFNα - + - + - + - +
OAS3
β -actin

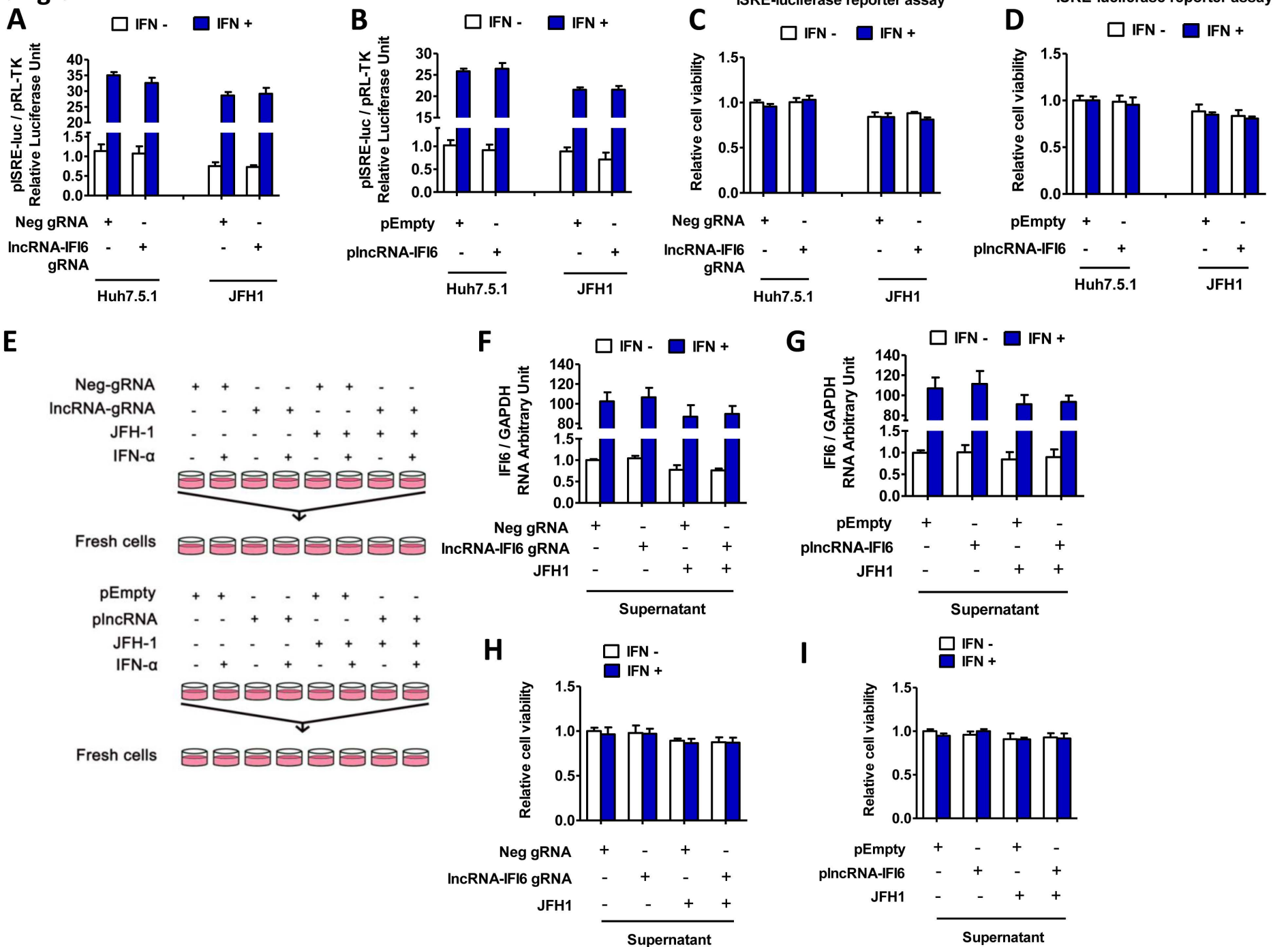
SFig 6.


A
IFN -
IFN +
Relative cell viability
Neg siRNA
IFI6 siRNA
JFH1
B
IFN -
IFN +
Relative cell viability
pEmpty
pIFI6
JFH1
C
IFN -
IFN +
JFH-1 / GAPDH RNA Arbitrary Unit
Neg siRNA
IFI6 siRNA
JFH1
D
IFN -
IFN +
IFI6 / GAPDH RNA Arbitrary Unit
Neg siRNA
IFI6 siRNA
JFH1
E
Neg siRNA
IFI6 siRNA
JFH1
IFNα
HCV core
IFI6
β -actin
F
Neg gRNA
lncRNA-IFI6 gRNA
Relative cell viability
Neg siRNA
IFI6 siRNA
JFH1
G
pEmpty
plncRNA-IFI6
Relative cell viability
pEmpty
pIFI6
JFH1
H
Neg gRNA
lncRNA-IFI6 gRNA
JFH-1 / GAPDH RNA Arbitrary Unit
Neg siRNA
IFI6 siRNA
JFH1
I
Neg gRNA
lncRNA-IFI6 gRNA
IFI6 / GAPDH RNA Arbitrary Unit
Neg siRNA
IFI6 siRNA
JFH1
J
Neg gRNA
lncRNA-IFI6 gRNA
Neg siRNA
IFI6 siRNA
JFH1
HCV core
IFI6
β -actin
K
pEmpty
plncRNA-IFI6
IFI6 / GAPDH RNA Arbitrary Unit
pEmpty
pIFI6
JFH1
L
Neg gRNA
lncRNA-IFI6 gRNA
JFH-1 / GAPDH RNA Arbitrary Unit
Neg siRNA
IFI6 siRNA
JFH1-PHH
M
Neg gRNA
lncRNA-IFI6 gRNA
IFI6 / GAPDH RNA Arbitrary Unit
Neg siRNA
IFI6 siRNA
JFH1-PHH
N
pEmpty
plncRNA-IFI6
JFH-1 / GAPDH RNA Arbitrary Unit
pEmpty
pIFI6
JFH1-PHH
O
pEmpty
plncRNA-IFI6
IFI6 / GAPDH RNA Arbitrary Unit
pEmpty
pIFI6
JFH1-PHH
P
IFN -
IFN +
lncRNA-IFI6 / GAPDH RNA Arbitrary Unit
Neg siRNA
IFI6 siRNA
JFH1
Q
IFN -
IFN +
lncRNA-IFI6 / GAPDH RNA Arbitrary Unit
pEmpty
pIFI6
JFH1

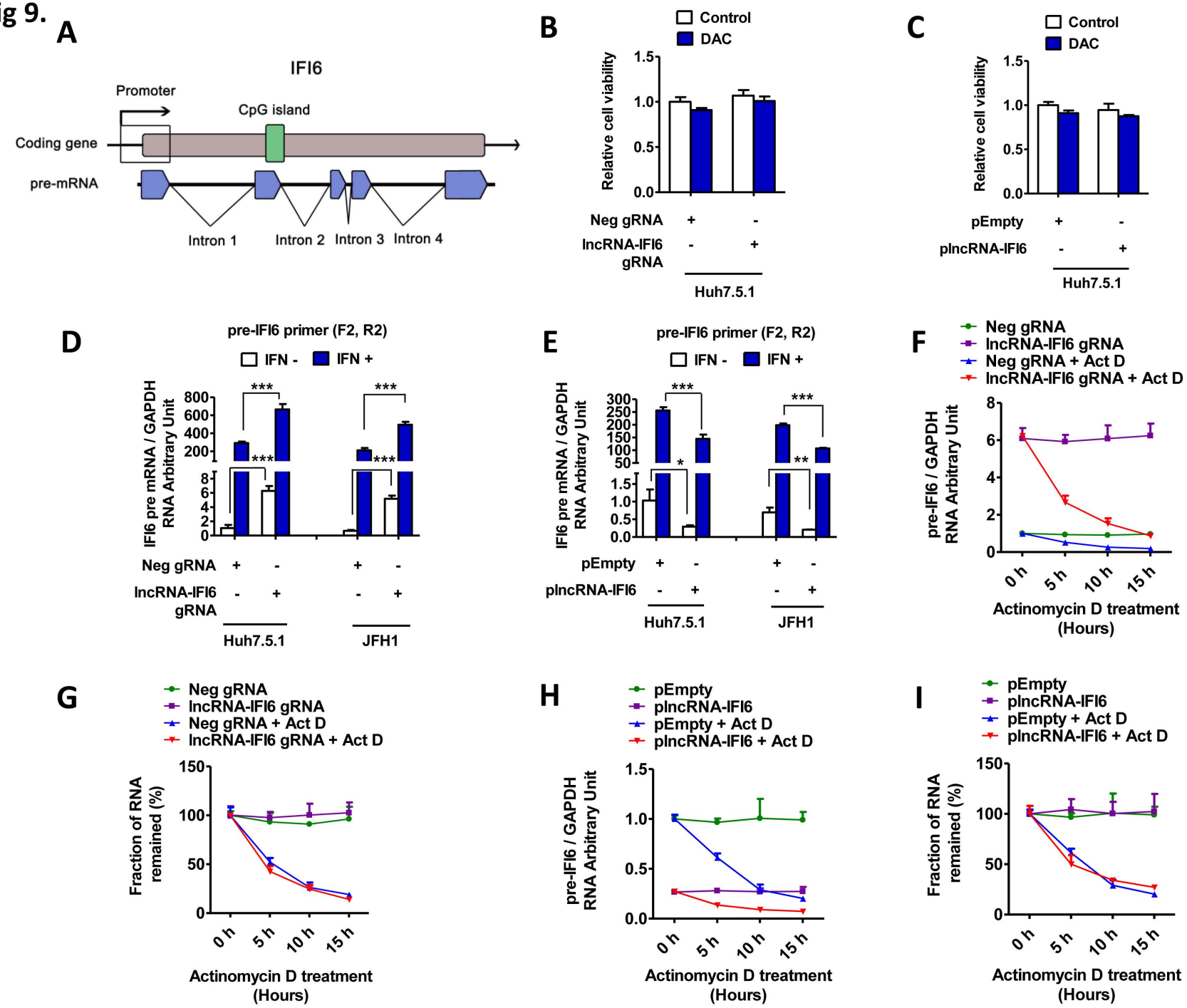
SFig 7.


A
IFN -
IFN +
STAT1 / GAPDH RNA Arbitrary Unit
Neg gRNA
lncRNA-IFI6 gRNA
Huh7.5.1
JFH1
B
Neg gRNA
lncRNA-IFI6 gRNA
JFH1
IFNα
STAT1
β -actin
C
IFN -
IFN +
STAT1 / GAPDH RNA Arbitrary Unit
pEmpty
plncRNA-IFI6
Huh7.5.1
JFH1
D
pEmpty
plncRNA-IFI6
JFH1
IFNα
STAT1
β -actin
E
IFN -
IFN +
lncRNA-IFI6 / GAPDH RNA Arbitrary Unit
Neg siRNA
STAT1 siRNA
STAT2 siRNA
JAK1 siRNA
Huh7.5.1
F
IFN -
IFN +
JAK1 / GAPDH RNA Arbitrary Unit
Neg siRNA
JAK1 siRNA
Huh7.5.1
G
IFN -
IFN +
STAT1 / GAPDH RNA Arbitrary Unit
Neg siRNA
STAT1 siRNA
Huh7.5.1
H
IFN -
IFN +
STAT2 / GAPDH RNA Arbitrary Unit
Neg siRNA
STAT2 siRNA
Huh7.5.1
I
IFN -
IFN +
Relative cell viability
Neg siRNA
STAT1 siRNA
STAT2 siRNA
JAK1 siRNA
Huh7.5.1

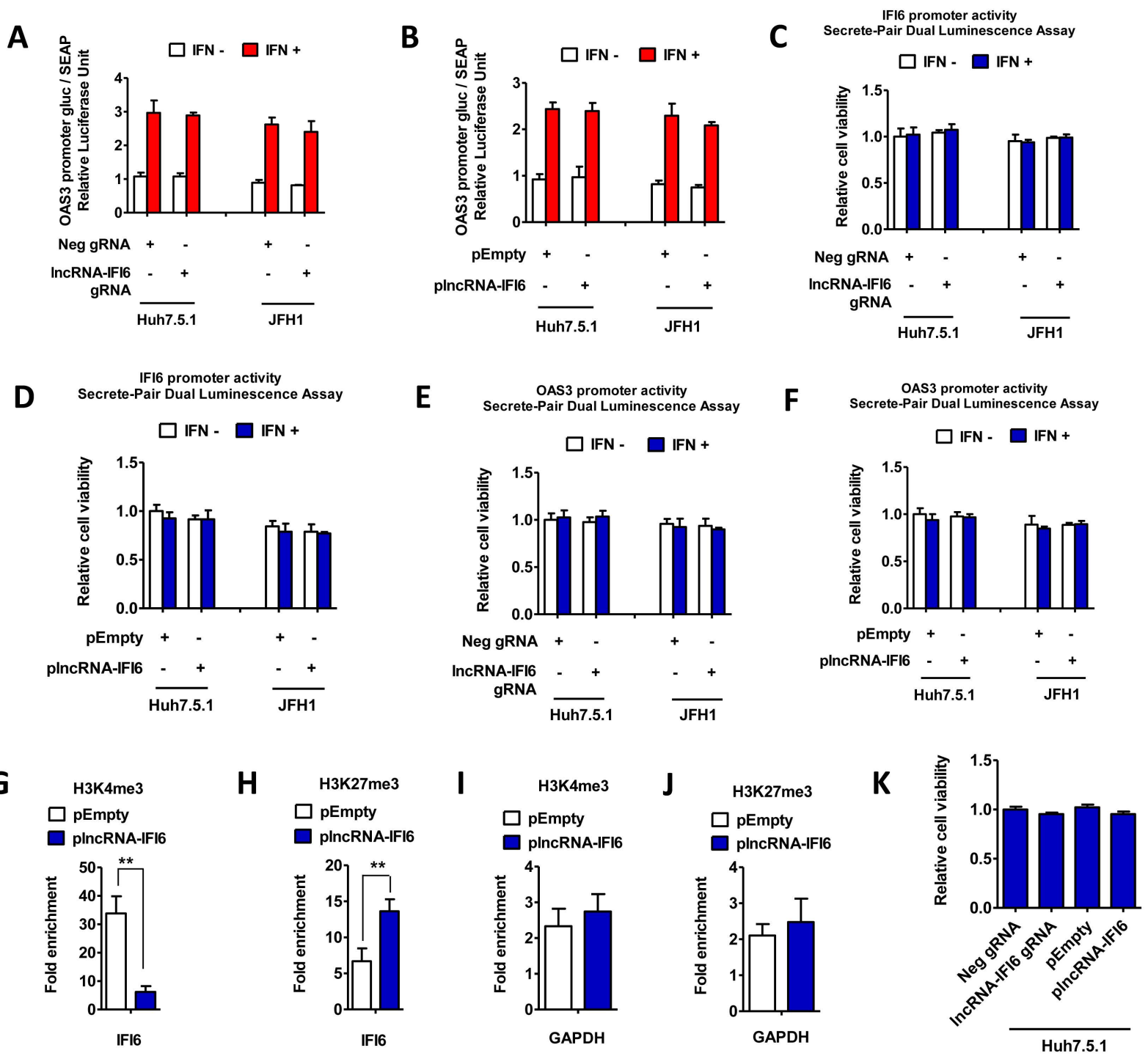
SFig 8.


A
IFN -
IFN +
pISRE-luc / pRL-TK Relative Luciferase Unit
Neg gRNA
lncRNA-IFI6 gRNA
Huh7.5.1
JFH1
B
pEmpty
plncRNA-IFI6
C
ISRE-luciferase reporter assay
Relative cell viability
D
E
Neg-gRNA
lncRNA-gRNA
JFH-1
IFN-α
Fresh cells
pEmpty
plncRNA
F
IFI6 / GAPDH RNA Arbitrary Unit
JFH1
Supernatant
G
H
I

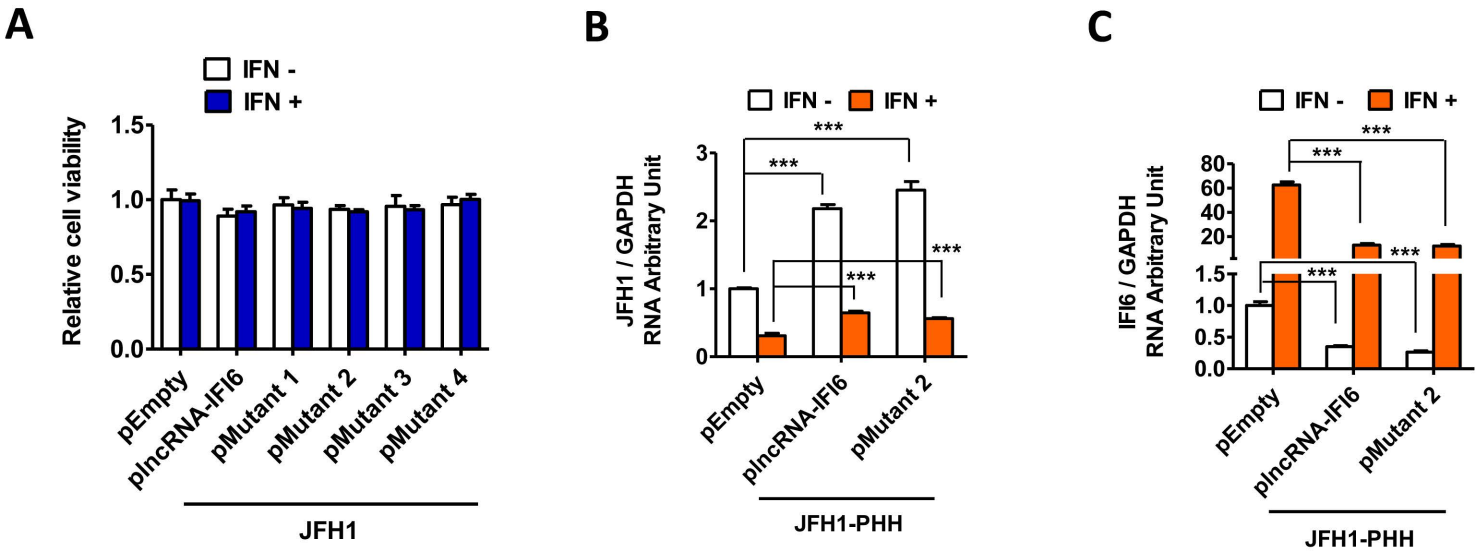
SFig 9.


A
IFI6
Promoter
CpG island
Coding gene
pre-mRNA
Intron 1
Intron 2
Intron 3
Intron 4
B
Control
DAC
Relative cell viability
Neg gRNA
lncRNA-IFI6 gRNA
Huh7.5.1
C
Control
DAC
Relative cell viability
pEmpty
plncRNA-IFI6
Huh7.5.1
D
pre-IFI6 primer (F2, R2)
IFN -
IFN +
IFI6 pre mRNA / GAPDH RNA Arbitrary Unit
Neg gRNA
lncRNA-IFI6 gRNA
Huh7.5.1
JFH1
E
pre-IFI6 primer (F2, R2)
IFN -
IFN +
IFI6 pre mRNA / GAPDH RNA Arbitrary Unit
pEmpty
plncRNA-IFI6
Huh7.5.1
JFH1
F
Neg gRNA
lncRNA-IFI6 gRNA
Neg gRNA + Act D
lncRNA-IFI6 gRNA + Act D
pre-IFI6 / GAPDH RNA Arbitrary Unit
Actinomycin D treatment (Hours)
G
Neg gRNA
lncRNA-IFI6 gRNA
Neg gRNA + Act D
lncRNA-IFI6 gRNA + Act D
Fraction of RNA remained (%)
Actinomycin D treatment (Hours)
H
pEmpty
plncRNA-IFI6
pEmpty + Act D
plncRNA-IFI6 + Act D
pre-IFI6 / GAPDH RNA Arbitrary Unit
Actinomycin D treatment (Hours)
I
pEmpty
plncRNA-IFI6
pEmpty + Act D
plncRNA-IFI6 + Act D
Fraction of RNA remained (%)
Actinomycin D treatment (Hours)

SFig 10.


A
IFN -
IFN +
OAS3 promoter gluc / SEAP
Relative Luciferase Unit
Neg gRNA
lncRNA-IFI6 gRNA
Huh7.5.1
JFH1
B
IFN -
IFN +
OAS3 promoter gluc / SEAP
Relative Luciferase Unit
pEmpty
plncRNA-IFI6
Huh7.5.1
JFH1
C
IFI6 promoter activity
Secrete-Pair Dual Luminescence Assay
IFN -
IFN +
Relative cell viability
Neg gRNA
lncRNA-IFI6 gRNA
Huh7.5.1
JFH1
D
IFI6 promoter activity
Secrete-Pair Dual Luminescence Assay
IFN -
IFN +
Relative cell viability
pEmpty
plncRNA-IFI6
Huh7.5.1
JFH1
E
OAS3 promoter activity
Secrete-Pair Dual Luminescence Assay
IFN -
IFN +
Relative cell viability
Neg gRNA
lncRNA-IFI6 gRNA
Huh7.5.1
JFH1
F
OAS3 promoter activity
Secrete-Pair Dual Luminescence Assay
IFN -
IFN +
Relative cell viability
pEmpty
plncRNA-IFI6
Huh7.5.1
JFH1
G
H3K4me3
pEmpty
plncRNA-IFI6
Fold enrichment
**
IFI6
H
H3K27me3
pEmpty
plncRNA-IFI6
Fold enrichment
**
IFI6
I
H3K4me3
pEmpty
plncRNA-IFI6
Fold enrichment
GAPDH
J
H3K27me3
pEmpty
plncRNA-IFI6
Fold enrichment
GAPDH
K
Relative cell viability
Neg gRNA
lncRNA-IFI6 gRNA
pEmpty
plncRNA-IFI6
Huh7.5.1

SFig 11.


A
IFN -
IFN +
Relative cell viability
1.5
1.0
0.5
0.0
pEmpty
pIncRNA-IFI6
pMutant 1
pMutant 2
pMutant 3
pMutant 4
JFH1
B
IFN -
IFN +
JFH1 / GAPDH
RNA Arbitrary Unit
3
2
1
0
***
***
***
***
pEmpty
pIncRNA-IFI6
pMutant 2
JFH1-PHH
C
IFN -
IFN +
IFI6 / GAPDH
RNA Arbitrary Unit
80
60
40
20
1.5
1.0
0.5
0.0
***
***
***
***
pEmpty
pIncRNA-IFI6
pMutant 2
JFH1-PHH