

POSTER PRESENTATION

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Transcriptional regulation of IFN- λ genes in Hepatitis C virus-infected hepatocytes via IRF-3·IRF-7·NF- κ B complex

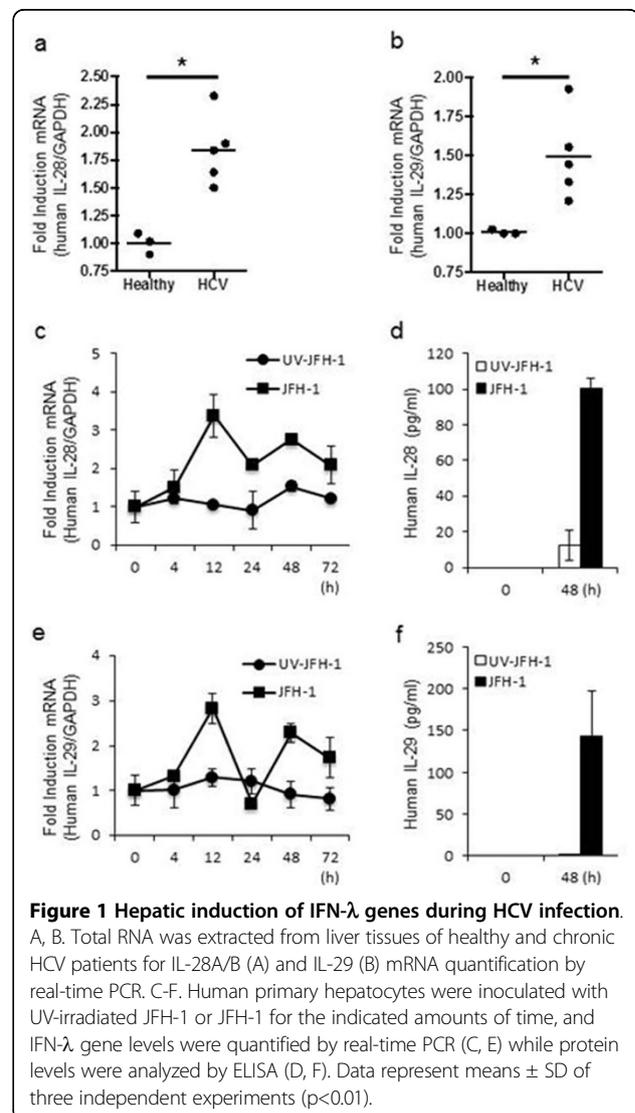
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From Society for Immunotherapy of Cancer 29th Annual Meeting
National Harbor, MD, USA. 6-9 November 2014

Hepatitis C virus (HCV) infection in hepatocytes stimulates innate antiviral responses including the production of type III interferons (IFN- λ), including IL-28A, IL-28B, and IL-29 (Figure 1). However, the molecular mechanism(s) regulating the expression of IFN- λ genes in HCV-infected hepatocytes remains undefined. In this study, we examined regulatory elements involved in the induction of IFN- λ genes following HCV infection in hepatocytes and further determined the binding of specific transcription factor(s) to promoter regions of IFN- λ genes. Our studies reveal that the regulatory portion for IL-28A, IL-28B, and IL-29 genes is localized to a 1-kb region in their respective promoters (Figure 2, 4). Notably, interferon regulatory factor (IRF)-3 and -7 are the key transcriptional factors for the induction of IL-28A and IL-28B genes (Figure 5, 6), whereas NF- κ B is an additional requirement for the induction of the IL-29 gene (Figure 3). Ligation of Toll-like receptors (TLR) 3, 7, 8, and 9, which also activate IRFs and NF- κ B, resulted in more robust production of IFN- λ than that observed with HCV infection, verifying the importance of TLR pathways in IFN- λ production (Figure 8). Furthermore, the addition of IFN- λ to HCV-infected hepatocytes decreased viral replication and produced a concurrent reduction in microRNA-122 (miR-122). The decrease in viral replication was enhanced by the co-administration of IFN- λ and miR-122 inhibitor (miRIDIAN) (Figure 7), suggesting that such combinatorial therapies may be beneficial for the treatment of chronic HCV infection.

Published: 6 November 2014

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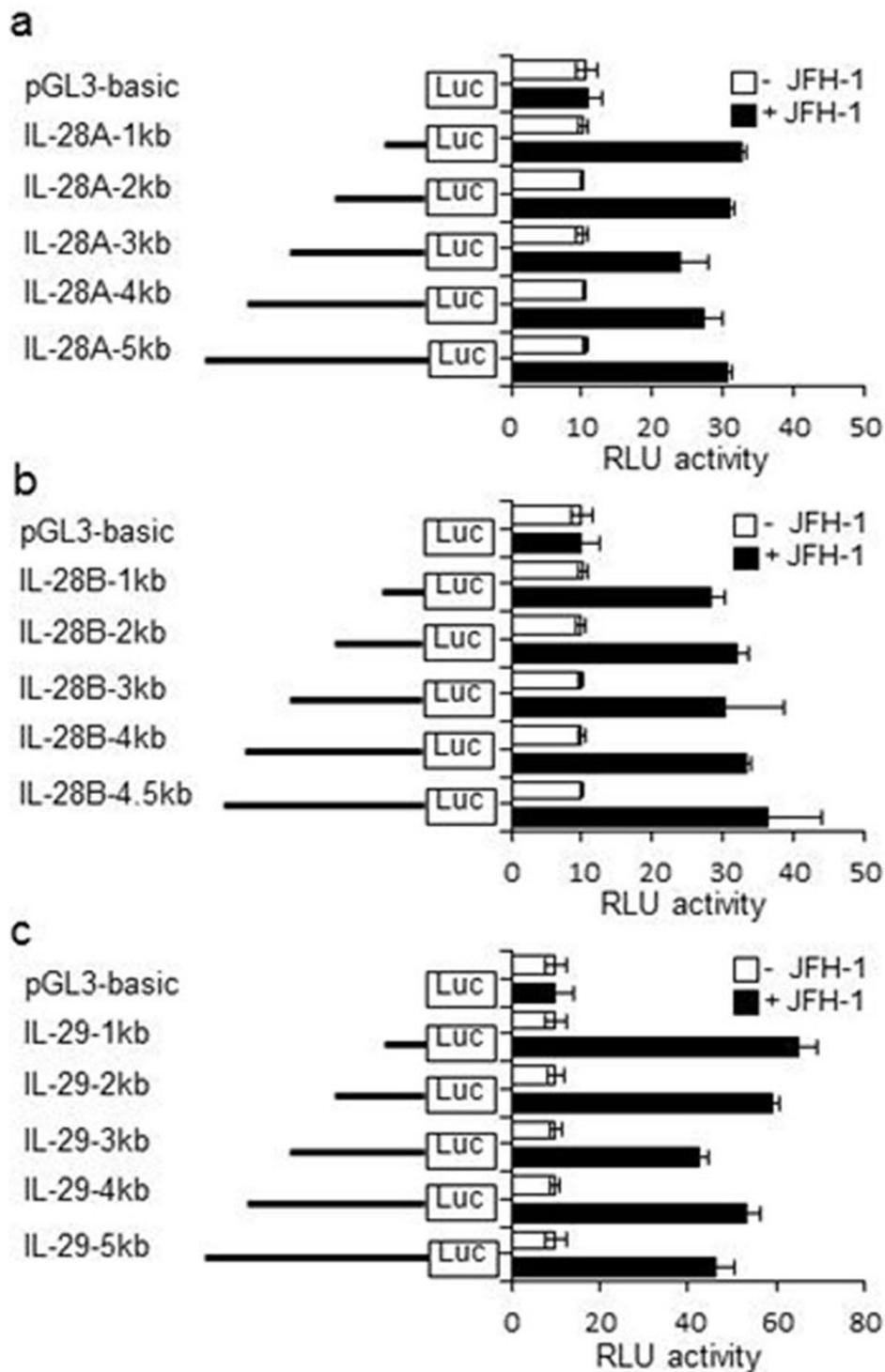


Figure 2 Transcriptional activity at the IL-28A, IL-28B, and IL-29 promoter by HCV infection. PH5CH8 cells were transfected with a mixture of the luciferase constructs containing various 5' deletions of the human IL-28A (A), IL-28B (B), or IL-29 (C) promoters and Renilla luciferase control vector. The cells were cultured for 18 hours and then infected with JFH-1 for 12 hours. Luciferase activity in whole cell lysates was normalized to Renilla luciferase activity. Data are the mean \pm SD of triplicate data points from five independent experiments.

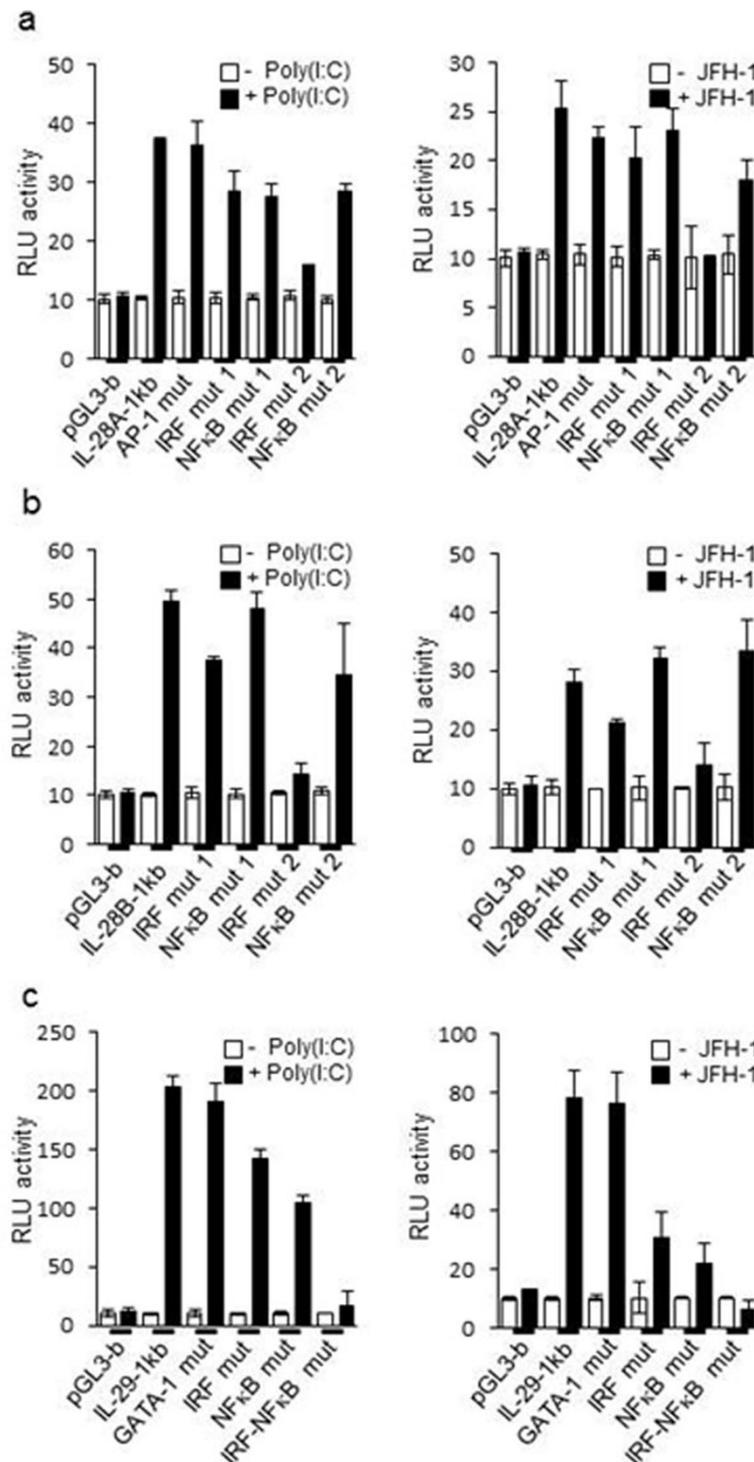


Figure 3 IRF-7, and NF- κ B differentially determine expression of IL-28A/B and IL-29. Nucleotide sequence from 1.0 kb upstream of the transcription start site of human IL-28A, IL-28B, and IL-29. The positions of the IRF, NF- κ B, AP-1, and GATA-1 binding sites are indicated. (B-D) PH5CH8 cells were transiently transfected with control plasmid pGL3-basic, and plasmids encoding luciferase constructs containing human IL-28A (pGL3-IL-28A 1kb) (B), IL-28B (pGL3-IL-28B 1kb) (C), or IL-29 (pGL3-IL-29 1kb) (D) promoter regions. Various mutation constructs of the above promoter regions were also transfected into PH5CH8 cells, using a Renilla luciferase vector as a control. The cells were cultured for 24 hours and then stimulated with poly(I:C) for 6 h or infected with JFH-1 for 12 h. Luciferase activity in whole cell lysates was normalized to Renilla luciferase activity. Data are the mean \pm SD of triplicate data points from five independent experiments.

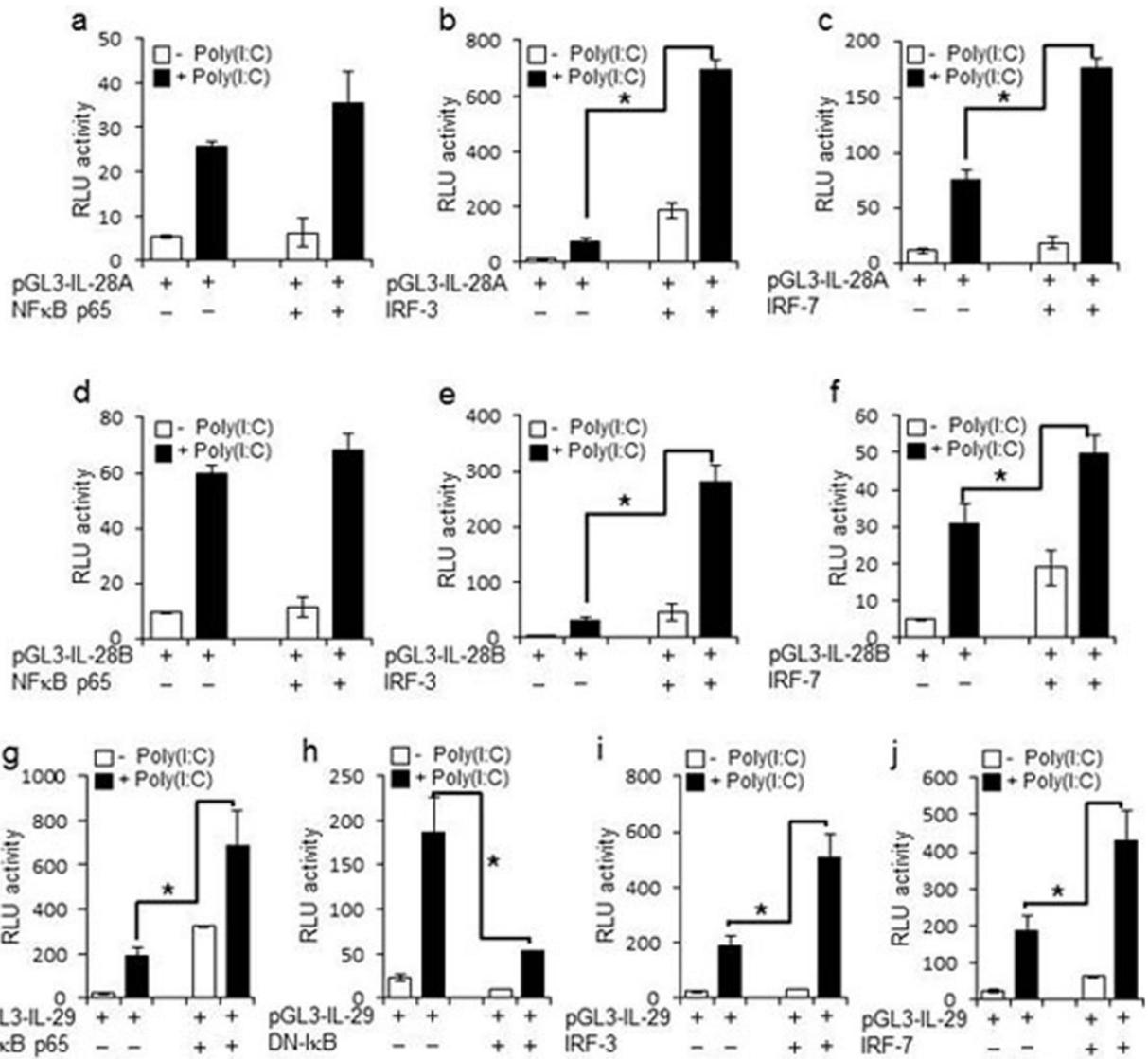


Figure 4 Overexpression of IRFs and NF-κB confirms their role in expression of IFN-λ genes. PH5CH8 cells were transiently transfected with plasmids encoding different promoter regions of human IL-28A (pGL3-IL-28A 1kb) (A-C), IL-28B (pGL3-IL-28B 1kb) (D-F), or IL-29 (pGL3-IL-29 1kb) (G-J), and expression vectors encoding IRF-3, IRF-7, and NF-κB or a dominant-negative mutant of IKKβ (DN-κB). At 18 h post-transfection, cells were stimulated with poly(I:C) for 6 hours. Luciferase activity in whole cell lysates was normalized to Renilla luciferase activity. Data represent mean ± SD of triplicate data points of one experiment and are representative of five independent experiments (p<0.01).

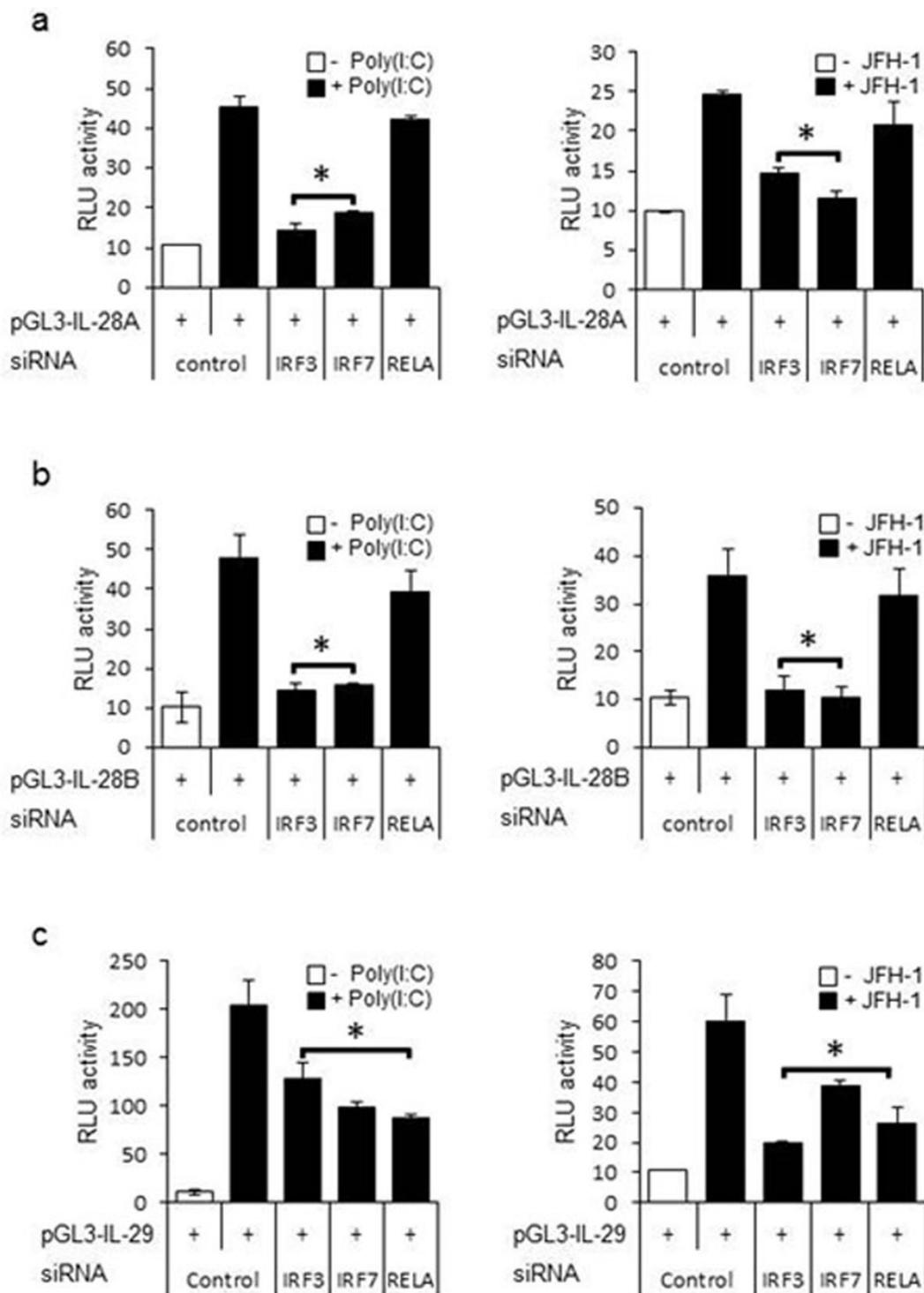


Figure 5 IRFs are core transcription factors of IFN- λ genes. IL-28B (B), or IL-29 (C) in combination with siRNA specific for IRF-3, IRF-7, RELA (NF- κ B p65), or control. At 36 h post-transfection, cells were stimulated with poly(I:C) for 6 hours or infected with JFH-1 for 12 h. Luciferase activity in whole cell lysates was normalized to Renilla Luciferase activity. Data represent mean \pm SD of triplicate data points of one experiment and are representative of three independent experiments. Statistical significance ($p < 0.01$) is relative to control + poly(I:C) or control + JFH-1.

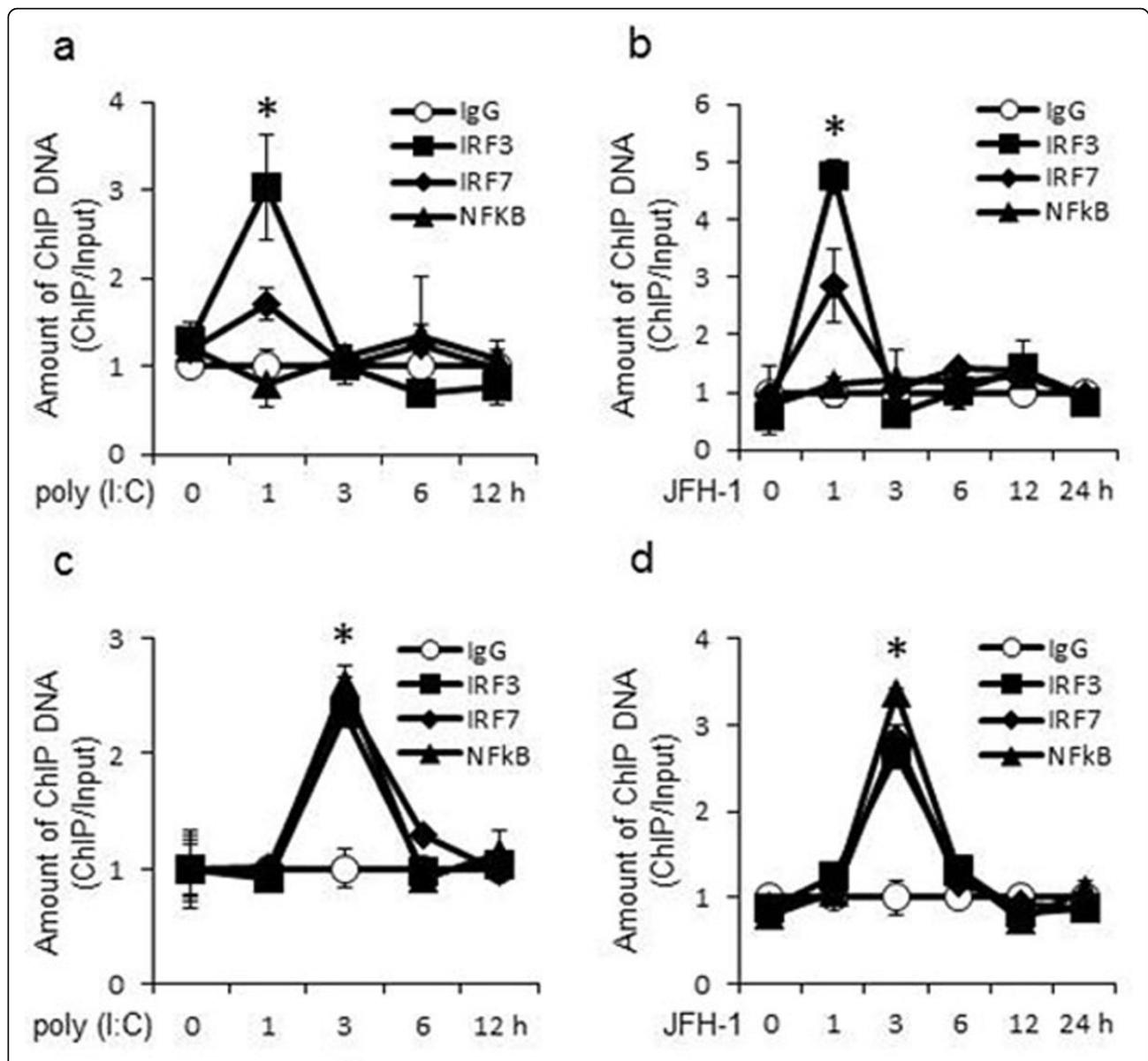
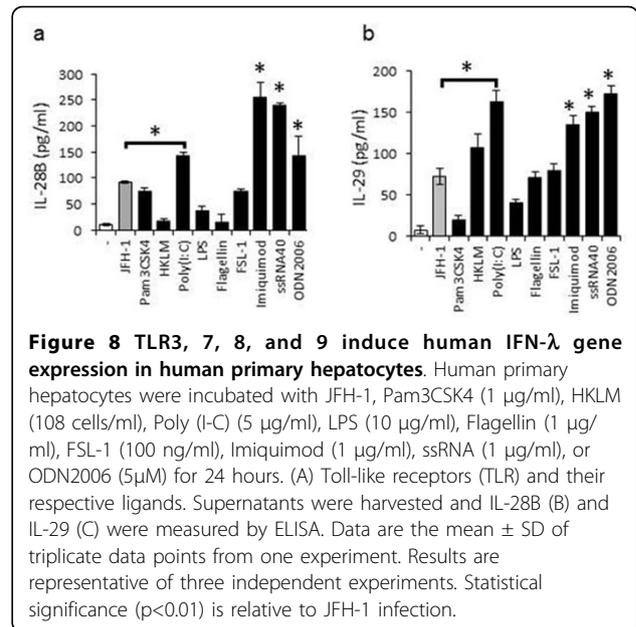
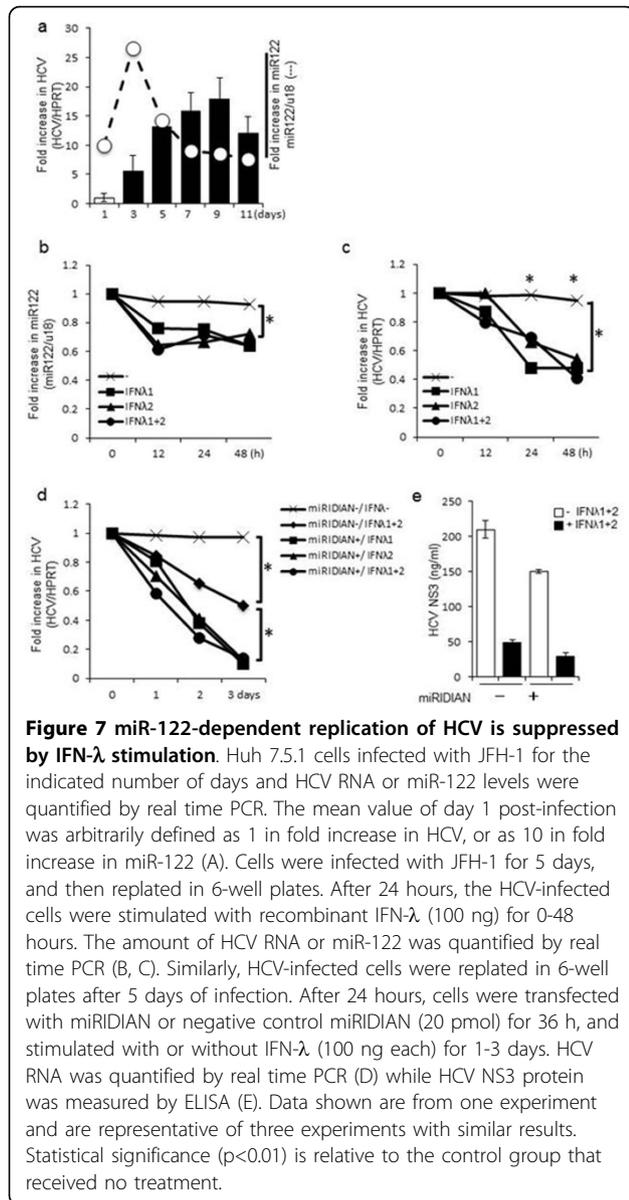


Figure 6 IRFs and NF- κ B directly bind the promoters of IFN- λ genes. PH5CH8 cells were stimulated with poly(I:C) (A and C) or infected with JFH-1 (B and D) for the indicated lengths of time. Chromatin was immunoprecipitated with anti-IRF-3, anti-IRF-7, anti-NF- κ B antibody or control normal rabbit IgG. Purified ChIP and input DNA were analyzed by real-time quantitative PCR. The amount of ChIP DNA was normalized to that of input DNA. The mean value of control antibody before stimulation was arbitrarily defined as 1. Data are the mean \pm SD of triplicate data points from a representative of five independent experiments. Statistical significance ($p < 0.01$) is relative to IgG.



doi:10.1186/2051-1426-2-S3-P173

Cite this article as: Lee *et al.*: Transcriptional regulation of IFN-λ genes in Hepatitis C virus-infected hepatocytes via IRF-3-IRF-7-NF-κB complex. *Journal for ImmunoTherapy of Cancer* 2014 **2**(Suppl 3):P173.

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