Hepatitis C virus (HCV) interacts extensively with host factors to not only establish productive infection but also trigger unique pathological processes. Our recent genome-wide siRNA screen demonstrated that IκB kinase-α (IKK-α) is a crucial host factor for HCV. Here we describe a new nuclear factor κB (NF-κB)-independent and kinase-mediated nuclear function of IKK-α in HCV assembly. HCV, through its 3′ untranslated region, interacts with DEAD box polypeptide 3, X-linked (DDX3X) to activate IKK-α, which translocates to the nucleus and induces a CBP/p300-mediated transcriptional program involving sterol regulatory element-binding proteins (SREBPs). This innate pathway induces lipogenic genes and enhances core-associated lipid droplet formation to facilitate viral assembly. Chemical inhibitors of IKK-α suppress HCV infection and IKK-α–induced lipogenesis, offering a proof-of-concept approach for new HCV therapeutic development. Our results show that HCV uses a novel mechanism to exploit intrinsic innate responses and hijack lipid metabolism, which may contribute to high chronicity rates and the pathological hallmark of steatosis in HCV infection.

HCV infection is a leading cause of chronic liver disease associated with substantial morbidity and mortality worldwide. A protective HCV vaccine is not available, and the current therapeutic regimen is suboptimal. The virus has a unique propensity to cause persistent infection and induce progressive liver damage. HCV extensively exploits host factors, such as cellular lipid metabolic pathways, for efficient propagation. HCV has been shown to alter the lipid metabolism of infected hepatocytes, resulting in a unique pathological feature of HCV infection: hepatic steatosis. Activation of SREBPs, which are crucial transcriptional regulators of cholesterol and fatty acid metabolism, has been shown in HCV-infected hepatocytes. However, the mechanistic basis of their activation remains unclear.

HCV activates host innate immunity that functions to limit viral infection. Recognition of viral pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), such as RIG-I–like receptors (RLRs), and activation of various signaling pathways, including interferon regulatory factor 3 (IRF3) and NF-κB, are early steps of an intrinsic innate immune response that leads to the subsequent induction of interferons. The NF-κB pathway is tightly regulated by the IKK complex, which consists of two catalytic subunits, IKK-α and IKK-β, and a regulatory subunit, NEMO (also known as IKK-γ). Activation of IKK-β and NEMO and subsequent IκB degradation are crucial steps in the activation of the canonical NF-κB pathway. IKK-α preferentially phosphorylates NF-κB2 rather than IκB and leads to the activation of p52-RelB heterodimers that regulate a distinct subset of NF-κB target genes. This alternative action is referred to as the noncanonical pathway. IKK-α is a remarkably versatile molecule that is involved in diverse signaling pathways to regulate gene expression; many of its actions are independent of NF-κB. Unlike IKK-β, IKK-α can shuttle between the cytoplasm and the nucleus. In the nucleus, IKK-α interacts with CREB binding protein (CBP) and contributes to NF-κB–mediated gene expression through phosphorylation of histone H3.

RESULTS

IKK-α is required for productive HCV infection

We previously used a two-part viral infection protocol to characterize host dependencies associated with both the early (part-one) and late (part-two) stages of the HCV life cycle. We confirmed that the effect of IKK-α silencing was more pronounced in the part-two (~85% inhibition) than the part-one assay (~60% inhibition) in HuH7.5.1 cells, suggesting that IKK-α acts more during the late stage of viral infection (Fig. 1a and Supplementary Fig. 1a). We confirmed the effect of IKK-α depletion by testing four individual siRNAs of the SMARTpool (Fig. 1b and Supplementary Fig. 1b). Expression of a siRNA-resistant IKK-α mutant restored HCV infection in cells treated with siRNA targeting IKK-α (Supplementary Fig. 1c), further validating the phenotype-specific role of IKK-α in HCV infection.
IKK-α siRNA substantially impaired the production and secretion of infectious HCV by more than 90% (Fig. 1c). Overexpression of IKK-α by transfecting Huh7.5.1 cells with a plasmid encoding hemagglutinin-tagged IKK-α (HA–IKK-α) substantially increased infectious HCV production (Fig. 1d). The function of IKK-α requires its kinase activity, and the kinase-defective K44M mutant of IKK-α (called here IKK-αKM) behaves as a dominant-negative mutant. Transfer of the IKK-αKM blocked HCV propagation similarly to IKK-α siRNA (Fig. 1e).

To further investigate the role of IKK-α in HCV infection, we tested various IKK inhibitors in HCV-infected Huh7.5.1 cells and primary human hepatocytes (PHHs) (Fig. 1f–i and Supplementary Fig. 2). Treatment of Huh7.5.1 cells with wederolactone and IKK Inhibitor XII, which are chemical inhibitors of both IKK-α and IKK-β, markedly reduced HCV core protein staining and infectious viral particle production (Fig. 1g). Increasing concentrations of both compounds led to a dose-dependent decline in HCV RNA production and secretion in both Huh7.5.1 cells and PHHs that could not be accounted for by a minor cytotoxicity at high concentrations (Fig. 1h,i and Supplementary Fig. 2b,c). BMS-345541, an IKK-β-specific inhibitor, had little effect on HCV production (Fig. 1f–i and Supplementary Fig. 2b,c).

The role of IKK-α in HCV assembly and lipid droplet formation

To investigate the step of the HCV life cycle in which IKK-α is required, we used multiple virologic assays. IKK-α silencing preferentially affected extracellular HCV RNA levels in the HCV cell culture infection system (Fig. 1b). We therefore specifically examined single-cycle replication by transfecting genomic HCV RNA into CD81-deficient Huh7 (Huh7.25) cells and found that HCV replication was not affected by IKK-α silencing (Fig. 2a). IKK-α silencing had no effect on assays targeting individual steps of the early HCV life cycle, including entry (HCV pseudovirus assay), translation (HCV internal ribosome entry site (IRES)-driven reporter) and replication (subgenomic replicon) (Fig. 2b and Supplementary Fig. 3), consistent with a pre-dominant role of IKK-α in the late stage of the viral life cycle.

We thus hypothesized that IKK-α is involved in the assembly or secretion of infectious viral particles. The lipid droplet has been shown to play a crucial part in HCV assembly22–24. Consistent with this, we observed HCV proteins, particularly the core and NS5A proteins, in close proximity to lipid droplets in HCV-infected cells (Fig. 2c and Supplementary Fig. 4a,b). Cells infected with HCV had a marked increase in lipid droplet numbers, lipid droplet–positive area, lipid droplet fluorescence intensity and triglyceride content, and IKK-α silencing significantly blocked this increase (Fig. 2c and Supplementary Fig. 4b,c). In HCV-infected cells treated with IKK-α siRNA, core protein staining was only modestly reduced, but the association between core protein and lipid droplets was diminished substantially (Fig. 2c and Supplementary Fig. 4b), suggesting that IKK-α is important for the association of lipid droplets with HCV proteins during the assembly process. The IKK inhibitors wederolactone and Inhibitor XII, but not BMS-345541, showed strong inhibition of lipid droplet formation.
**Figure 2** The function of IKK-α in HCV assembly and HCV-induced lipid droplet formation. (a) Effects of various siRNAs on HCV JFH-1/P7-luciferase (Luc) RNA replication in CD81-deficient Huh7.25 cells. (b) Effects of various siRNAs on HCV subgenomic replicon assay.

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and colocalization of core protein and lipid droplets (Supplementary Fig. 4d). IKK-α overexpression also significantly increased the number, size and fluorescence intensity of lipid droplets (Fig. 2d). Conversely, transfection of the HA–IKK-αEM strongly reduced lipid droplet formation (Fig. 2d). Collectively these data indicate that IKK-α predominantly affects HCV-induced lipid droplet formation and viral assembly, although an additional effect on virion secretion cannot be ruled out completely.

**HCV 3′ UTR activates IKK-α and lipid droplet formation**

To define the mechanism of the HCV-responsive activation of IKK-α and induction of lipogenesis, we examined the role of the 3′ UTR of the viral genome, which contains a previously identified HCV PAMP molecule: a poly(U/C) sequence. Transfection of HCV 3′ UTR RNA demonstrated a strong induction of lipid droplet formation, and IKK-α silencing by siRNA markedly diminished the HCV 3′ UTR–mediated increase in lipid droplet content (Fig. 2e and Supplementary Fig. 5a). Poly(I:C), a synthetic PAMP, also enhanced lipid droplet formation (Supplementary Fig. 5a).

Transfection of HA–IKK-α plasmid and HCV 3′ UTR RNA together resulted in increased amounts of phosphorylated IKK-α, whereas we did not detect IKK-α phosphorylation in cells transfected with the HA–IKK-αEM (Supplementary Fig. 5b). Therefore HCV infection induces lipid droplet biogenesis through viral RNA–triggered activation of an IKK-α–dependent pathway.

The function of IKK-α in HCV infection is NF-κB independent

IKK-α is a component of IKK that controls NF-κB activation, but it has also been implicated in NF-κB–independent functions. To dissect the function of the NF-κB pathway in connection with IKK-α in HCV infection, we first silenced four major factors of the NF-κB family (NF-κB1, NF-κB2, RELA and RELB) and two IKK catalytic units essential for NF-κB activation (IKK-β and NEMO) (Fig. 3a–c). We observed an increase in HCV infection and replication (Fig. 3a,b), which directly contrasts the result of IKK-α silencing. Silencing of the above-mentioned NF-κB genes did not affect IKK-α–mediated induction of lipid droplet formation or enhancement of HCV propagation (Fig. 3d,e). In addition, signals known to activate the NF-κB pathway, such as interleukin-1β (IL-1β) and lymphotoxin-α and -β (LT-α/β), did not induce lipid droplet formation (Fig. 3f and Supplementary Fig. 5c). siRNA against LT-β (also called LTB) or its receptor (LTBR), which are known to be involved in noncanonical activation of NF-κB through IKK-αEM, had little or no effect on HCV replication or lipid droplet induction (Supplementary Fig. 5d–g). Further evidence for the NF-κB–independent effect of IKK-α is shown in the Supplementary Results. In addition, several previously reported NF-κB–independent functions of IKK-α are not involved in modulating HCV infection (Supplementary Results).

The role of DDX3X in HCV assembly and lipid droplet formation

We next explored the signaling pathway that mediates the specific effect of the HCV 3′ UTR on IKK-α activation. RIG-I has been shown to be the key PRR for the HCV PAMP that resides in the 3′ UTR. Because RIG-I is nonfunctional in Huh7.5.1 cells, we tested the roles of two other RLRs (MDA5 (also known as IFI17) and LGP2...
In HCV-infected cells, we verified increased interaction of DDX3X with IKK-α using coimmunoprecipitation (Fig. 4f). Silencing of DDX3X markedly reduced the colocalization of the HCV 3′ UTR with IKK-α, whereas IKK-α siRNA had no effect on complex formation between the HCV 3′ UTR and DDX3X (Supplementary Fig. 7a), suggesting that interaction of the HCV 3′ UTR with DDX3X is required for the subsequent recruitment and activation of IKK-α. Silencing of MAVS, which has been suggested to interact with DDX3X in the activation of innate immunity28,32, had no effect on interactions among the HCV 3′ UTR, DDX3X and IKK-α (Supplementary Fig. 7a). These data indicate that in HCV-infected Huh7.5.1 cells, DDX3X signals predominantly through the IKK-α–mediated lipogenic pathway to play a proviral part in HCV propagation.

Previous studies showed that the HCV core binds and redistributes DDX3X to the viral assembly sites around lipid droplets; however, this interaction seems to be dispensable for HCV replication33. In HCV-infected cells, redistributed DDX3X partially colocalized with core and lipid droplets (Supplementary Fig. 8f). Treatment of Huh7.5.1 cells with the HCV 3′ UTR or poly(I:C) also induced DDX3X–IKK-α association, but the DDX3X–IKK-α complexes did not localize to the lipid droplets (Fig. 4g and Supplementary Fig. 8e). To confirm that the core is not involved in the interaction between DDX3X and IKK-α, we studied an HCV mutant with a core amino acid substitution (Y35A) that abolishes its interaction with DDX3X33 and showed that in the absence of core-DDX3X binding, DDX3X–IKK-α interaction is still induced by HCV infection without lipid droplet association (Fig. 4h and Supplementary Fig. 8g).

HCV induces IKK-α phosphorylation and nuclear translocation

We next examined the consequence of DDX3X–IKK-α interaction induced by HCV infection. We infected Huh7.5.1 cells with HCV and harvested them at various time points (4–72 h) after infection to determine IKK-α gene expression. There was no change in either mRNA or protein levels of IKK-α in the presence of HCV infection (Supplementary Fig. 9a,b). However, we did find a significant increase in phosphorylated IKK-α, representing the active form of IKK-α,
at 12–24 h after HCV infection (Fig. 5a and Supplementary Fig. 9c). IKK-α, when activated, shuttles from the cytoplasm to the nucleus. We therefore examined the distribution of IKK-α in the nuclear and cytosolic fractions of HCV-infected cells. By 12 h after HCV infection, the cells showed marked nuclear accumulation of IKK-α (Fig. 5b). Treatment of cells with tumor necrosis factor-α (TNF-α), a bona fide activator of IKK-α, significantly enhanced the phosphorylation and nuclear translocation of IKK-α, whereas the overall expression of IKK-α remained unchanged (Fig. 5a,b and Supplementary Fig. 9b). Confocal microscopy confirmed the nuclear accumulation of IKK-α in HCV-infected or HCV 3′ UTR–transfected cells (Fig. 5c and Supplementary Fig. 9d,e), and these cells showed a speckle-like association of IKK-α and DDX3X (Fig. 5c) and elevated lipid droplet contents (Supplementary Fig. 9e,f), whereas the majority of IKK-α resided in the cytoplasm of uninfected cells. Phosphorylation of IKK-α is required for its nuclear translocation, as the HA–IKK-αM did not show nuclear localization (Supplementary Fig. 9g).

IKK-α induces SREBP-mediated lipid droplet formation

We performed microarray gene expression profiling and identified cellular genes that are transcriptionally regulated by IKK-α. We treated Huh7.5.1 cells with either nontargeting control siRNA or IKK-α siRNA in the absence or presence of HCV infection (four conditions total). We confirmed the knockdown efficiency of IKK-α and its effect on HCV infection by measuring IKK-α mRNA and HCV RNA levels (Supplementary Fig. 10a,b). Lipogenic genes, particularly SREBPs and SREBP target genes involved in fatty acid and triglyceride synthesis, were upregulated by HCV, and their inductions were abrogated in IKK-α–silenced cells (Fig. 5d and Supplementary Fig. 10c–i). Overexpression of wild-type (WT) IKK-α upregulated, and overexpression of the IKK-αKM downregulated, the expression of SREBPs (Supplementary Fig. 10j). Under our experimental conditions, the effects of HCV infection and transfection tended to be underestimated, as the efficiencies of infection and transfection were only ~40–50% of cells under the best circumstance.

We compared our microarray data set with a recent publication that identified all SREBP-1–regulated lipid metabolism and human hepatic genes. We showed that the genes regulated by SREBP-1 were markedly affected by HCV infection or IKK-α silencing (Supplementary Fig. 10k,l), implying that IKK-α has a major role in SREBP-1 regulation of hepatic lipid metabolism genes. We further demonstrated that...
SREBP knockdown has a similar effect as IKK-α silencing on HCV infection. After depletion of SREBP-1 and SREBP-2, there was a significant impairment of HCV production (Fig. 5e), likely stemming from the apparent diminution of lipid droplet formation (Fig. 5f). IKK-α silencing and IKK inhibitors (wedelolactone and Inhibitor XII) also abrogated HCV 3′ UTR–induced SREBP induction (Fig. 5g).

**Figure 5** HCV infection, IKK-α activation, SREBP induction and lipid droplet formation. (a) Effect of HCV infection or TNF-α treatment on the phosphorylation of IKK-α (pIKK-α) as determined by immunoprecipitation followed by western blotting. h.p.i., hours post infection. (b) The nuclear-to-cytoplasm ratio of IKK-α determined by dividing the relative intensity of nuclear IKK-α normalized to β-tubulin (TFIIB) over that of cytoplasmic IKK-α normalized to β-tubulin. (c) Immunostaining for DDX3X and IKK-α in Huh7.5.1 cells treated with the HCV 3′ UTR or full-length (FL) HCV RNA for 4 h. White arrows indicate nuclear translocation of IKK-α in viral RNA–treated cells showing colocalization of IKK-α and DDX3X. Scale bars, 20 μm. (d) SREBP mRNA levels in IKK-α-deficient cells measured at 72 h after siRNA treatment, 24 h after HCV infection or after both treatments and normalized relative to siNT-treated samples in the absence of HCV infection. (e,f) Effects of SREBP silencing in Huh7.5.1 cells on HCV infection (e) and lipid droplet formation (f). Scale bars, 20 μm. (g) Effects of various IKK inhibitors (wedelolactone, 30 μM; IKK Inhibitor XII, 10 μM; or BMS-345541, 1 μM) on HCV 3′ UTR–mediated induction of SREBP-1. Error bars represent ± s.d. of triplicate experiments (d,e,g). *P < 0.05, **P < 0.01 determined by Student’s t test. NS, not significant.

**Figure 6** The signaling pathway involved in IKK-α–mediated lipogenic induction of HCV infection. (a) Effects of IKK-α or CBP/p300 siRNA on SREBP luciferase reporter activities. (b) Lipid droplet contents and HCV core expression in CBP or p300 siRNA–treated Huh7.5.1 cells before HCV infection. Scale bars, 20 μm. (c) Effects of CBP/p300 silencing on HCV infection. Left, intracellular and extracellular HCV RNA levels; right, HCV core quantification in part one and part two of the HCV cell culture assay. A western blot analysis of CBP and p300 protein levels is shown in Supplementary Figure 12c. (d) ChIP assays performed with the indicated antibodies and Huh7.5.1 cells that were untreated (ctrl), infected with HCV or transfected with HCV 3′ UTR RNA for 48 h. Only data for the SREBP-1 promoter are shown here, and data for the IL-8 (positive control) and actin (negative control) promoters are shown in Supplementary Figure 12d. Data are shown as the mean ± s.d. of triplicate experiments. Pol II, polymerase II. (e) IKK-α overexpression in HCV-infected cells deprived of SREBP-1, SREBP-2 or CBP/p300. (f) A proposed model of the innate antiviral response, HCV-induced lipogenesis and lipid droplet formation in HCV assembly. The thickness of the arrows represents the putative magnitude of the two pathways (proviral or antiviral) in Huh7.5.1 cells. Dashed arrows represent possible crosstalk between the two parallel pathways. Error bars represent ± s.d. of triplicate experiments (a,c,e). *P < 0.05, **P < 0.01 compared to control determined by Student’s t test.
and Supplementary Fig. 10i). In PHHs, IKK inhibitors reduced the mRNA levels of SREBP-1 and SREBP-2 (Supplementary Fig. 10m). Silencing of DDX3X, IKK-α, SREBP-1 or SREBP-2 in PHHs led to a substantial reduction of extracellular as compared to intracellular HCV RNA levels (Supplementary Fig. 11a,b).

**IKK-α induces expression of SREBP through CBP/p300**

Activated IKK-α has been shown to phosphorylate CBP and upregulates its activities in the nucleus\(^1\). CBP/p300 is also involved in SREBP transcriptional activity\(^35,36\). Overexpression of CBP/p300 markedly upregulates the expression of both SREBP-1 and SREBP-2 (ref. 37). We found that both IKK-α and CBP/p300 siRNAs significantly reduced SREBP promoter activities and mRNA levels (Figs. 5d and 6a and Supplementary Fig. 11c,d). We also found that IKK-α exerts its effect on SREBP induction and lipogenesis through recruiting the CBP/p300 complex. CBP/p300 depletion by siRNA significantly decreased cytosolic lipid droplet content and HCV production (Fig. 6b,c). siRNAs against CBP/p300 markedly diminished HCV 3′ UTR–mediated SREBP-1 induction and lipid droplet formation (Supplementary Fig. 11d,e). Using chromatin immunoprecipitation (ChIP) assays, we found that bindings of the SREBP-1 promoter to both IKK-α and CBP were enhanced by HCV infection or HCV 3′ UTR transfection (Fig. 6d and Supplementary Fig. 11f). In addition, enhanced viral production by IKK-α overexpression was substantially compromised in cells treated with CBP/p300 or SREBP siRNA (Fig. 6e), indicating that CBP/p300 and SREBPs are involved in the IKK-α–mediated proviral effect.

To show that the DDX3X–IKK-α–SREBP pathway is indeed crucial for productive HCV infection, we silenced these genes in combination and demonstrated that HCV infection was much more reduced (>100-fold) than when silencing the individual genes (Supplementary Fig. 11g).

**DISCUSSION**

We demonstrate a previously unrecognized function of IKK-α in regulating cellular lipid metabolism and therefore HCV assembly, both of which are independent of its role in intrinsic innate immunity. We extensively validated and mechanistically investigated this function of IKK-α. We showed that HCV, through the action of the viral 3′ UTR containing a previously identified PAMP, specifically interacts with DDX3X and activates IKK-α to mediate an NF-κB–independent function in the nucleus. Recent accumulating evidence has indicated an emerging role of DDX3X in innate immunity, independent function in the nucleus. Recent accumulating evidence has indicated an emerging role of DDX3X in innate immunity, although the precise mechanism remains unclear\(^28,29\). Other members of the DEAD box family, including the well-known RLRs and their activation by HCV further stimulates the lipogenic pathways to facilitate viral assembly. We reason that IKK-α and its associated pathways have a dual effect on HCV infection—an antiviral effect through the NF-κB pathway and a proviral effect through the lipogenic pathway—with the latter being the dominant pathway in our experimental models. IKK-α signaling may therefore be a pivotal mechanism whereby chronic infection and inflammation lead to the dysregulated metabolism that is implicated in the development of various chronic metabolic disorders\(^52\). Our findings offer crucial insights into the inflammatory origin of metabolic disease and the pathogenic consequences of chronic viral infection\(^14,53\).

A comprehensive understanding of the crosstalk between HCV, host innate responses and lipid metabolism may help identify new and broadly active antiviral targets\(^54\). The discovery of a proviral function of IKK-α offers therapeutic opportunities for IKK-α inhibitors in the treatment of HCV infection. The demonstrated efficacy of commercially available inhibitors of IKK activities in blocking HCV infection in this study provides a proof-of-concept approach for new HCV therapeutic development. We also present the first evidence, to our knowledge, that HCV usurps intrinsic innate immune pathway for its own advantage and survival. Such an exploitation of host antiviral
defense may underlie the mechanism whereby HCV infection predisposes to persistent infection despite an active host immunity.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** Microarray data have been deposited in the Gene Expression Omnibus under accession code GSE46528.

**Note:** Supplementary information is available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

QL and TJL conceived and designed the study. QL, V.P, S.K. and H.C. conducted experiments. Q.L., V.P and T.J.L. analyzed data. Q.L. and T.J.L. wrote the paper with the input from V.P. T.J.L. supervised the studies.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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ONLINE METHODS

siRNA transfection. siRNAs were transfected into Huh7.5.1 cells at a final concentration of 50 nM using a reverse transfection protocol with Oligofectamine (Invitrogen) as previously described54. For PHHs (provided by the NIH-funded Liver Tissue Procurement and Cell Distribution System, N01-DK-7-0004/IHSN267007/00004C), cells were seeded on 12-well plates at 500,000 cells per well and transfected with siRNA at a final concentration of 50 nM using RNAiMAX (Invitrogen). Unless otherwise indicated, further treatments or assays were typically performed 72 h after siRNA transfection, when gene silencing efficiency reaches its maximal level.

HCV core staining during part one (early stage) and part two (late stage). For part one, Huh7.5.1 cells were treated with the indicated SMARTpool siRNAs at a concentration of 50 nM for 72 h and then infected with the HCV JFH-1 strain. After 48 h, cells were stained and imaged for HCV core production. This detects host factors involved in the early stages of the viral life cycle, comprising entry, viral protein translation and RNA replication. Culture supernatants of part-one cells were transfected and used to infect naïve Huh7 cells, thus starting part two, which detects proteins involved in the later stages of viral infection, including virion assembly and release. siRNAs against CD81 and ApoE served as proviral controls for part one and part two, respectively. A detailed protocol of the core staining is described in the Supplementary Methods.

In vitro transcription and labeling of HCV RNA and transfection. Plasmids carrying JFH1, J6/JFH1 or Luc-JFH1/P7 genomic RNA or subgenomic replicon RNA sequences were linearized with XbaI and purified by phenol-tetrachloroform-isooamyl alcohol extraction. In vitro transcription was performed using the MEGAscript T7 kit (Ambion) according to the manufacturer’s protocol. The quality and quantity of RNA were evaluated by NanoDrop spectrophotometer (Thermo Scientific). Aliquots (22 l, 1 µg ml−1) of RNA were stored frozen at −80 °C until use. HCV 3′ UTR RNA was generated from a plasmid harboring the 3′ UTR of HCV that contains a poly(U/C) region previously defined as the viral PAMP25. Cy3 labeling of RNA was conducted by using the Silencer siRNA labeling kit Cy3 (Ambion). Biotin-labeled RNA was generated by including biotin-UTP (Roche; 1:6 ratio of biotin to UTP) during the synthesis process. RNA transfection was performed using DMRIE-C Reagent (Invitrogen) according to the manufacturer’s instructions.

HCV life-cycle assays. HCV life-cycle assays were performed using HCV pseudoparticles (for viral entry) and subgenomic replicons (for viral IRES-mediated translation and RNA replication), and single-cycle infection assays were conducted using CD81-deficient Huh7-25 cells transfected with JFH1/P7-Luc or J6/JFH1 RNA. Detailed protocols for the various assays are described in the Supplementary Methods.

Immunofluorescence, lipid staining, confocal microscopy and quantification of images. Cells grown on Lab-Tek II borosilicate four-well chamber cover slips (Nunc) were fixed with 4% paraformaldehyde, permeabilized in 0.3% Triton X-100 and incubated with blocking solution in PBS containing 3% BSA and 10% normal goat serum (Vector Laboratories). Cells were then labeled with the appropriate primary antibodies diluted in PBS with 1% BSA followed by incubation with Alexa Fluor 488, 568 or 647-conjugated secondary antibodies (Invitrogen) in PBS with 1% BSA. The primary antibodies used were to the following: core (generated from anti-core 6G7 hybridoma cells, 1:500), NSSA (9E10, a gift of C. Rice of the Rockefeller University, 1:1,000), NS5B (ab65410, Abcam, 1:500), N5 (abs 65407, Abcam, 1:200), HA (C2994, Cell Signaling, 1:500), IKK-α (ab54626, Abcam, 1:200) and DDX3X (A300-474A, Bethyl Laboratories, 1:250). Nuclei were counterstained with Hoechst 33342 (Invitrogen) at 1:5,000 in PBS. Lipid droplets were stained with BODIPY 493/503 (Invitrogen) applied at 1 µg ml−1 for 1 h in PBS with 1% BSA. Each step was followed by three washes with PBS. Confocal laser scanning microscopy analysis was performed with an Axio Observer.Z1 microscope equipped with a Zeiss LSM 5 Live Duoscan System under an oil immersion 1.4 numerical aperture (NA) ×63 objective lens (Carl Zeiss). Images were acquired using ZEN 2009 software (Carl Zeiss). Dual or triple color images were acquired by consecutive scanning with only one laser line active per scan to avoid cross excitation. Quantification of lipid droplets was performed with ImageJ (US National Institutes of Health), ZNF2009 and a set of defined intensity thresholds that were applied to all images.

Microarray analysis. Huh7.5.1 cells were transfected with nontargeting or IKK-α siRNA for 72 h and then mock infected or infected with HCV at a multiplicity of infection (MOI) of 1. Forty-eight hours later, cellular RNA was extracted and purified using RNeasy Mini Kit (Qiagen). RNA was quantified with a spectrophotometer, and RNA quality was analyzed with an Agilent Bioanalyzer according to the manufacturer’s instructions. RNA was then amplified with an Agilent Enzo kit. Amplified complementary RNA was hybridized to an Affymetrix Human 133 Plus 2.0 microarray chip containing 54,675 gene transcripts. Microarray analyses were performed at the NIDDK Microarray Core Facility. The bioinformatics and statistical analyses were described previously55. A >1.5-fold change in expression combining a >95% probability of being differentially expressed (P < 0.05) was considered to be biologically significant. The infectivity of the JFH-1 virus for Huh7.5.1 cells was tested before the microarray experiment.

Gene expression assay. Total cellular RNA from a replica experiment was prepared with the RNeasy Mini Kit (Qiagen). CDNA was synthesized from total RNA with the First Strand cDNA Synthesis Kit (Roche). The mRNA expression levels of target genes were quantified by quantitative PCR using gene-specific primers and probes (IDT) and TaqMan Gene Express Master Mix (Applied Biosystems) on an ABI 7500 Real Time PCR System. Relative transcript levels were calculated using the ΔΔCT method with 18S ribosomal RNA as the normalizing control gene.

ChIP assays. ChIP assays were performed using the SimpleChIP Enzymatic Chromatin IP Kit (#9003, Cell Signaling) as described by the manufacturers. In brief, after crosslinking, nuclei were purified, and chromatin was sheared by sonication (three times, 20 s each). Chromatin was incubated overnight with specific antibodies against IKK-α (ab4111, Abcam, 1:500), CBP (ab2832, Abcam, 1:500) and RNA polymerase II CTD (MA1-46093, Thermo Scientific, 1:500). Isotype IgG was used as a negative control for the immunoprecipitation experiments. Immunoprecipitated chromatin was then incubated with protein G magnetic beads, washed and eluted. After reversal of the crosslinks and purification of DNA, precipitated DNAs were analyzed by quantitative real-time PCR (40 cycles) with specific primers to the human actin promoter as a negative control, the IL-8 promoter as a positive control37 and the SREBP-1 promoter (5′-GCTGTCGGCTTTATAGGCTTT-3′ and 5′-TCTACCGGGGAGTGAGAGGGA-3′). Quantitative real-time PCR was performed in duplicate on duplicate ChIP assays using 500 nM of the above oligonucleotide primers and input DNA standards diluted in fivefold increments from 5% to 0.008% with SYBR Green PCR Master Mix (#4309155, Applied Biosystems) and the Applied Biosystems 7500 Sequence Detection System. The fold increases of the associations of IKK-α, CBP and Pol II to the IL-8 and SREBP-1 promoters were normalized to actin signals and calculated for HCV-infected and HCV PAMP RNA-transfected cells as compared to untreated cells. P value was calculated using Student’s t-test.

Chemical inhibitor studies. IKK Inhibitors II (wedelolactone), XII and III (BMS-345541) were purchased from Merck (EMD) Chemicals. Serial dilutions of the IKK inhibitors were made in 100% DMSO immediately before the assay so that the final concentrations of DMSO in each reaction were identical. Cells were infected with JFH-1 at an MOI of 0.5 for 6 h and then treated with fresh DMEM containing DMSO with or without IKK inhibitors. Cultures were incubated for 48 h, and HCV RNA was extracted from the cell lysates or culture medium and quantified by TaqMan real-time PCR. The experiments were done in triplicate, and each dose was tested at least twice.

Additional methods. Detailed methodology is described in the Supplementary Methods.

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Corrigendum: Hepatitis C virus infection activates an innate pathway involving IKK-α in lipogenesis and viral assembly

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In the version of this article initially published, the HCV core mutant (F24Y) virus used in Figure 4h and Supplementary Figure 8g and attributed as a gift from A. Patel was incorrectly named. The correct name of the virus is Y35A. The error has been corrected in the HTML and PDF versions of the article.