The triglyceride-synthesizing enzyme acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) plays a critical role in hepatitis C virus (HCV) infection by recruiting the HCV capsid protein core onto the surface of cellular lipid droplets (LDs). Here we find a new interaction between the non-structural protein NS5A and DGAT1 and show that the trafficking of NS5A to LDs depends on DGAT1 activity. DGAT1 forms a complex with NS5A and core and facilitates the interaction between both viral proteins. A catalytically inactive mutant of DGAT1 (H426A) blocks the localization of NS5A, but not core, to LDs in a dominant-negative manner and impairs the release of infectious viral particles, underscoring the importance of DGAT1-mediated translocation of NS5A to LDs in viral particle production. We propose a model whereby DGAT1 serves as a cellular hub for HCV core and NS5A proteins, guiding both onto the surface of the same subset of LDs, those generated by DGAT1. These results highlight the critical role of DGAT1 as a host factor for HCV infection and as a potential drug target for antiviral therapy.

Despite immense drug discovery efforts, chronic hepatitis C virus (HCV) infection remains a serious public health concern. More than 170 million people are infected worldwide, and persistent viral replication in these individuals fuels viral transmission and the risk of progressive liver diseases, including cirrhosis and hepatocellular carcinoma (1). There is no vaccine, and current treatment regimens remain very taxing and have limited efficacy against some viral strains (2).

HCV is a single positive-stranded RNA virus that is translated into a single polyprotein upon entry into host cells and is then co- and post-translationally cleaved into 10 individual viral proteins. Structural proteins, including the viral capsid core, and the envelope glycoproteins E1 and E2, form new virions, whereas nonstructural proteins NS2–5B form an RNA replicase complex necessary for viral RNA replication. NS5A is a membrane-associated phosphoprotein involved in these two critical functions, viral RNA replication and virion assembly, due to its interactions with cellular and viral factors, including the viral capsid core (3, 4).

In infected cells, NS5A localizes to the endoplasmic reticulum (ER), ER-derived membranes, termed the "membranous web" that host RNA replication complexes, and to cellular LDs; the latter is a critical step in encapsidation of viral RNA into newly formed progeny virions (5–7). LDs, long regarded as mere triglyceride storage compartments, have emerged as dynamic cellular organelles involved in storage, transport, and utilization of neutral lipids (8), which are hijacked by several microbes, including HCV (9). Triglycerides are generated by two cellular diacylglycerol acyltransferase (DGAT) enzymes, DGAT1 and DGAT2, and are thought to fuel LD formation at ER membranes where DGAT enzymes are located. Both enzymes are expressed in liver and are functionally redundant in their role as triglyceride-synthesizing enzymes in hepatocytes, whereas DGAT2 functions as the critical enzyme regulating general lipogenesis during development (10). We recently showed that the HCV core protein interacts with DGAT1 and depends on DGAT1 activity for access to LDs. Inhibition of DGAT1 activity or RNAi-mediated knockdown of DGAT1 severely impaired HCV infectious particle production, demonstrating DGAT1 is critical to HCV infection (11, 12).

Here we focus on the broader role of DGAT1 in HCV infection. We identify NS5A as a new binding partner of DGAT1 and demonstrate that DGAT1 interacts with both core and NS5A...
proteins and facilitates the binding of the two viral factors. Notably, NS5A depends on DGAT1 activity to localize to LDs, and disruption of NS5A loading to LDs with a dominant-negative mutant of DGAT1 significantly impacts virion production, highlighting the functional relevance of this new interaction between NS5A and DGAT1.

**EXPERIMENTAL PROCEDURES**

*Plasmids*—FLAG-tagged murine Dgat1 and Dgat2 expression constructs were generated as described (13). mDGAT1 point mutants and small hairpin RNA vectors targeting DGAT1 and DGAT2 were also generated as described (11). Luc-Jc1 reporter virus was described (14). The NS5A-GFP construct was generated by inserting GFP and Myc tags at the C-terminal end of the Genotype 1a NS5A open reading frame. FLAG-tagged HCV constructs were cloned from HCV JFH1 and purified by phenol-chloroform extraction. Lentivirus Production and Transduction—Lentiviral particles were produced as described (17). Infectious titers were determined by transducing cells with serial dilutions of the viral stocks and FACS analysis 2 days post-transduction. Transductions were done with 4 μg/ml Polybrene (Sigma) for 4 h at 37 °C.

*Immunoprecipitation, LD Isolation, and Western Blotting*—For immunoprecipitation experiments, cells were lysed in lysis buffer (150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 50 mM Tris HCl, pH 7.4, and protease inhibitor mixture (Sigma)) for 30 min at 4 °C and passed 10 times through a G23 needle. Clarified lysates were immunoprecipitated with α-FLAG M2-agarose (Sigma), α-HA HA-7 agarose (Sigma), or Protein A-agarose (Invitrogen), washed five times in lysis buffer, and resuspended in Laemmli buffer for SDS-PAGE.

For sequential immunoprecipitations, after the first immunoprecipitation with α-FLAG–agarose for 2 h, beads were washed 5 times, and then eluted with 10 μg of FLAG peptide in 100 μl of lysis buffer (F-3290, Sigma). The eluates were subjected to a second immunoprecipitation using α-HA–agarose, washed five times in lysis buffer, and resuspended in Laemmli buffer for SDS-PAGE.

LDs were isolated as described (5). Briefly, cells were scraped in PBS, resuspended in hypotonic buffer (50 mM HEPES, 1 mM EDTA, and 2 mM MgCl₂, pH 7.4) supplemented with protease inhibitors, and homogenized by applying 30 strokes in a tight-fitting Dounce homogenizer. After spinning for 5 min at 1500 rpm, post-nuclear fractions were mixed with equal volumes of 1.05 M sucrose in isotonic buffer (50 mM HEPES, 100 mM KCl, 2 mM MgCl₂) and placed at the bottom of SW55 Ti (Beckman) centrifuge tubes overlaid with isotonic buffer containing 1 mM PMSF and centrifuged for 2 h at 100,000 × g. Proteins from the floating LD fraction were precipitated with 15% trichloroacetic acid and 30% acetone, washed once with acetone, and resuspended in urea loading dye (200 mM Tris/HCl, pH 6.8, 8 M urea, 5% SDS, 1 mM EDTA, 0.1% bromphenol blue, 15 mM DTT). For Western blot analysis, cells were lysed using the lysis buffer described above for 30 min followed by SDS-PAGE. For chemiluminescent detection, we used ECL and ECL Hyperfilm (Amersham Biosciences).

In Vitro Transcription of HCV RNA and Transfection—Plasmids encoding HCV reporter viruses were linearized with PvuI and purified by phenol-chloroform extraction. In vitro transcription was carried out using the MegaScript T7 kit (Ambion) according to the manufacturer’s protocol. For RNA transfection, Huh7.5 cells were trypsinized, washed once in Opti-MEM and incubated with secondary antibodies for 30 min. For lipid staining experiments, fixed cells were stained for 20 min with HCS LipidTox Neutral Lipid Stains (Invitrogen), diluted 1000-fold in PBS solution. For colocalization quantification studies, we stained nuclei with Hoechst (Sigma).
Lipids were resuspended in 500 μl of H11003. The hexane:isopropyl alcohol was evaporated under nitrogen. Cells were lysed in 1/11003 buffer or fixed for immunostaining. On day 6, transfected cells were harvested on days 5 and 6 and used to infect naive Huh7.5 cells overnight. On day 5, aliquots were lysed for Western blot measurements. Cells infected with the luciferase reporter viruses were lysed in 1/2003 lysis buffer (Promega) for luciferase activity measurements. Cells infected with the luciferase reporter viruses were lysed in 1× lysis buffer (Promega). Luciferase activity was measured using the Luciferase Assay System (Promega) on a MonoLight 2010 Luminometer (Pegasus Scientific Inc.).

**RESULTS**

**NS5A Interacts with DGAT1**—To determine if DGAT1 has a broader role in HCV infection, we used co-immunoprecipitations (co-IPs) with endogenous DGAT1 and FLAG-tagged HCV proteins in Huh7 hepatoma cells. As expected DGAT1 associates with core. Interestingly, we also detected a new interaction with NS5A but not with E1, NS2, NS3, or NS4B proteins (Fig. 1A). To further characterize the association of DGAT1 and NS5A, we expressed FLAG-tagged DGAT1 or DGAT2 along with GFP-tagged NS5A or GFP alone in hepatoma cells. After immunoprecipitation of the FLAG-tagged DGAT enzymes, interaction with NS5A was detected with FLAG-DGAT1 but not with DGAT2. These results confirm a novel, specific interaction of NS5A and DGAT1 (Fig. 1B). Additionally, we confirmed this interaction in the context of HCV Jc1-infected cells (Fig. 1C). Here, we observed two bands, which likely correspond to the unphosphorylated and phosphorylated forms of NS5A, indicating that both forms interact with endogenous DGAT1. These data demonstrate that the interaction between NS5A and DGAT1 occurs during HCV infection.

**DGAT1 Acts as a Connector between NS5A and Core**—The interaction between NS5A and core is important for viral assembly (3, 18). As they both interact with DGAT1, we postulated that DGAT1 may bridge the interaction of the two viral proteins. In co-IP experiments with the HA-tagged core and GFP-tagged NS5A in 293T cells, we detected weak binding of NS5A to core (Fig. 2A, middle panel, middle lanes), which correlates with relatively low expression levels of DGAT1 in these cells (data not shown). Strikingly, the interaction was greatly enhanced when DGAT1 was co-expressed with NS5A (Fig. 2B, middle panel, middle lanes). This suggests that DGAT1 acts as a connector between NS5A and core, facilitating NS5A localization to lipid droplets.

**Triglyceride Extraction**—Hepatoma cells in 6-well plates were washed with PBS and incubated with 1 ml of hexane/isopropyl alcohol (3:2) on a vertical shaker at 100 rpm, 3× for 10 min. The hexane/isopropyl alcohol was evaporated under nitrogen. Lipids were resuspended in 500 μl of chloroform with 1% Trixon X-100, dried again, resuspended in 200 μl of water, mixed, and quantified with Infinity Triglycerides (Thermo Scientific, TR22421).

**Statistical Analysis**—Statistical analyses were performed using unpaired two-tailed Student’s t tests. Data in histograms are displayed as the means ± S.E.

**DGAT1 Localizes the HCV NS5A Protein to Lipid Droplets**

![Figure 1. NS5A interacts specifically with DGAT1.](http://www.jbc.org/)

**Figure 1.** NS5A interacts specifically with DGAT1. A, shown is co-immunoprecipitation of endogenous DGAT1 with FLAG-tagged HCV proteins core, E1, NS2, NS3, NS4B, and NS5A, in Huh7 cells. After immunoprecipitation with α-FLAG M2 affinity gel, the endogenous DGAT1 and FLAG-tagged proteins were detected by Western blots (WB) with respective antibodies. The input control was 12% of the whole-cell lysate used for each immunoprecipitation. NT, not transfected. B, shown is co-IP of NS5A-GFP in Huh7 cells transfected with expression vectors for FLAG-DGAT1, FLAG-DGAT2, NS5A-GFP, and GFP proteins. The input control was 15% of the whole-cell lysate used for each immunoprecipitation. C, co-IP of NS5A with endogenous DGAT1 from HCV Jc1-infected Huh7.5 is shown. Normal rabbit IgG (Invitrogen) was used as a negative control (−). The input control was 4% of the whole-cell lysate used for each immunoprecipitation.

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(Invitrogen), and resuspended in Cytomix buffer (120 mM KCl, 5 mM MgCl2, 0.15 mM CaCl2, 2 mM EGTA, 1.9 mM ATP, 4.7 mM GSH, 25 mM HEPES, 10 mM potassium phosphate buffer, pH 7.6) at 107 cells ml−1. 400 μl of the cell suspension was mixed with 10 μg of HCV RNA and pulsed at 260 V and 950 microfarads with the Gene Pulser II (Bio-Rad).

**HCV Production and Infection and Luciferase Analysis**—Huh7.5 cells were transfected with luciferase reporter HCV Luc-Jc1 as described above on day 1 and plated on 6-well plates. On days 2 and 3, they were transfected with 2 μg of plasmids and X-tremeGENE 9 DNA Transfection Reagent (Roche Applied Science). Media were changed on day 4. Supernatants were harvested on days 5 and 6 and used to infect naive Huh7.5 cells overnight. On day 5, aliquots were lysed for Western blot or fixed for immunostaining. On day 6, transfected cells were lysed in 1× lysis buffer (Promega) for luciferase activity measurements. Cells infected with the luciferase reporter viruses were lysed in 1× lysis buffer (Promega). Luciferase activity was measured using the Luciferase Assay System (Promega) on a MonoLight 2010 Luminometer (Pegasus Scientific Inc.).
DGAT1 Localizes the HCV NS5A Protein to Lipid Droplets

**A**

![Figure 2](image_url)

**B**

![Figure 2](image_url)

**C**

![Figure 2](image_url)

Enhanced when FLAG-DGAT1 was co-expressed (Fig. 2A, middle panel, left lanes), consistent with a model where DGAT1 binds both viral factors and strengthens their interaction. This model was supported by shRNA studies, in which knockdown of DGAT1, but not DGAT2, weakened the interaction of core and NS5A (Fig. 2, B and C).

Next, we performed sequential co-IPs in the transfected cells described above. First, we immunoprecipitated FLAG-tagged DGAT1 and eluted the associated proteins with an excess of FLAG peptide. We then incubated the eluates with HA beads to immunoprecipitate core and analyzed the double pulldown by Western blotting. We detected NS5A-GFP in cells expressing NS5A-FLAG, and HA-core is shown. Cell lysates and bound proteins were analyzed by Western blot with α-HA antibody-conjugated agarose and subjected to Western blotting. The input control was 12% of the whole-cell lysate used for the single immunoprecipitation. The control input was 12% of the whole-cell lysate used for each immunoprecipitation. C, shown is real-time RT-PCR analysis of DGAT1 or DGAT2 mRNA expression levels in HuH7 cells transduced with the corresponding shRNAs. Data are the mean ± S.E. n = 3 independent experiments.

**NS5A Association with LDs Depends on DGAT1 Activity**—NS5A localizes to LDs in infected cells (5, 7, 19) and in cells ectopically expressing only NS5A (20). We analyzed the localization of each HCV protein in hepatoma cells by epifluorescence microscopy and confirmed that only core and NS5A localized to LDs when expressed alone (Fig. 3A). To test whether DGAT1 activity is required for the association of NS5A with LDs, we treated HCV-infected hepatoma cells with two structurally unrelated DGAT1 inhibitors (iA and iB) (11, 16, 21) and examined NS5A localization to LDs. At concentrations that inhibited cellular DGAT1 activity and HCV production (iA and iB: 20 and 75 μM, respectively) (Fig. 3, B and C), we observed a marked reduction of NS5A at LDs, with a more diffuse, ER-like staining pattern (Fig. 4A). The percentage of NS5A colocalizing with LDs was 7-fold lower than that of cells treated with DMSO (Fig. 4B). Similar results were seen in hepatoma cells expressing tagged NS5A both by epifluorescence and confocal microscopy (Fig. 4, C–F). Thus, DGAT1 directly affects NS5A localization and not indirectly via core. We also confirmed our previous findings that DGAT1 inhibition, under normal cell culture conditions, does not reduce overall LD content in hepatoma cells, excluding the possibility that the loss of NS5A LD association in response to DGAT1 inhibitors is caused by an overall loss of LDs (Fig. 5D) (11).

The same results were obtained after isolation of LD fractions from hepatoma cells expressing FLAG-tagged NS5A. NS5A-FLAG protein was detected by Western blotting in the LD fraction from DMSO-treated, but not iA or iB-treated, cells (Fig. 5A). LD fractions contained similar amounts of LD-resident adipose differentiation-related (ADRP) protein and lacked the ER-resident protein calreticulin, demonstrating the absence of contaminating ER membranes. Treatment with exogenous fatty acids (oleate) did not increase NS5A association with LDs, suggesting that this process was maximally effective in hepatoma cells under basal conditions.

Similar results were obtained in HuH7 cells transduced with lentiviral vectors expressing specific shRNAs against DGAT1 or DGAT2. Both enzymes were knocked down by ~80% as determined by real-time RT-PCR (Fig. 5C) and Western blotting of DGAT1 (Fig. 5B). Next, we isolated LDs from shRNA-transduced cultures transfected with a construct expressing NS5A and found that NS5A was not detected in the LD fraction from DGAT1 knockdown cells, whereas its levels in the LD fraction of DGAT2 and control shRNA-transduced cells were similar (Fig. 5B). Collectively, these results demonstrate that the association of NS5A with LDs depends selectively on DGAT1. They also suggest that the enzymatic activity of DGAT1 is required and is likely to fuel LD generation at ER membranes close to DGAT1 that can be accessed by NS5A.

**Interaction of NS5A with Active DGAT1 Is Crucial for HCV Infectious Virions Production**—To demonstrate the functional relevance of the NS5A-DGAT1 interaction in the HCV life cycle, we used a catalytically inactive mutant of DGAT1.
This mutant interacted with NS5A with similar affinity as wild-type DGAT1 in co-IP assays (Fig. 6A), indicating the interaction does not require DGAT1 enzymatic activity, as reported for core (11). Interestingly, when overexpressed in hepatoma cells, DGAT1-H426A dominantly suppressed translocation of NS5A-GFP to LDs in cellular fractionation assays (Fig. 6B), whereas wild-type DGAT1 or DGAT2 did not. This effect was unique for NS5A; mutant DGAT1 did not prevent localization of core to LDs in parallel assays (Fig. 6C).

Microscopic analysis confirmed the colocalization of NS5A-GFP with DGAT1-H426A away from LDs in distinct subcellular microdomains, whereas most core protein remained localized at LDs, unaffected by mutant DGAT1 (Fig. 6, D and E). As observed in previous studies (10), wild-type DGAT1, unlike DGAT2, did not access LDs, suggesting that the interactions with the viral factor occurs only transiently at the ER.

Next, we tested the effect of overexpressing DGAT1-H426A on the production of infectious HCV particles. We transfected Huh7.5 hepatoma cells with HCV Luc-Jc1, a bicistronic clone of HCV expressing firefly luciferase, and constructs expressing wt FLAG-DGAT1, the H426A mutant, or an empty control vector. After transfection, cell supernatants were harvested and used to infect naive Huh7.5 cells (Infected), and the producing cells were lysed (transfected). Shown are luciferase values expressed as percentage of DMSO control (mean ± S.E., n = 3). NT, not treated.

**DISCUSSION**

Here, we report that DGAT1 interacts with the viral NS5A protein and identify DGAT1 as a cellular link between viral core and NS5A proteins, promoting their interaction. DGAT1, core,
and NS5A interact in one complex, which we propose is vital for localization of both viral proteins to LDs in infected cells. The finding that the catalytic activity of DGAT1 is critical for NS5A to access LDs supports the model that NS5A localizes to DGAT1-generated LDs in cells and that those LDs serve as assembly platforms in infected cells. Finally, evidence that impairment of NS5A trafficking to LDs affects viral production highlights the critical role of the new interaction between DGAT1 and NS5A in HCV infection (Fig. 8).

Based on data from previous publications, a model has been proposed in which LD-localized core recruits viral RNA replication complexes to the close vicinity of LDs through interactions with NS5A (3, 5). Our result that DGAT1 is a key determinant for the localization of NS5A to LDs in the absence of other viral components is in agreement with recent reports showing that the presence of other viral components (e.g. NS2 or core) modulates, but is not critical to the intracellular trafficking of NS5A (22, 23). Other non-structural viral proteins implicated in viral assembly (e.g. NS2 and p7) (24–26) do not localize to LDs in the absence of core or NS5A and do not interact with DGAT1 in our assays. These factors seem to be required at later stages in infectious particle production and regulate relocation of core from LDs to the ER as well as association of viral envelope protein E2 with viral factors, such as core and NS5A (24, 25).

A key finding that underscores the importance of the DGAT1-NS5A interaction for viral assembly is that catalytically inactive DGAT1 dominantly suppresses trafficking of viral RNA replication complexes to LDs, as shown in Fig. 4A and B. This suggests that DGAT1 is a critical factor for the viral RNA replication process to occur in the LD environment.

**FIGURE 4.** NS5A localization at LDs is dependent on DGAT1 activity. Shown are representative images (scale bar = 10 μm) and quantification of Huh7-Lunet cells transfected with HCV Jc1 RNA (A and B), NS5A-GFP expression vector (C and D), or NS5A-FLAG expression vector (E and F) and treated with DGAT1 inhibitors (IA at 20 μM or IB at 75 μM) or DMSO control for 48 h. Cells were fixed and stained with α-NS5A or α-FLAG antibodies (A and E, green), LipidTox Red (A and C) or far red (E) (LDs, red), and Hoechst (nucleus, blue). Green fluorescence in C arises directly from NS5A-GFP signal. Cells were analyzed by epifluorescence microscopy in A–D and by confocal microscopy in E–F. Colocalization of NS5A and LDs per cell was quantified by the automatic measurement program of the Zeiss axiovision software (mean of 40 cells ± S.E.) (B, D, and F).

**FIGURE 5.** NS5A association with LDs is dependent on DGAT1 activity. A, shown are Western blots of cell extracts (left panels) or isolated LD fractions (right panels) from Huh7-Lunet cells transfected with NS5A-GFP and incubated with oleate, DMSO, 20 μM DGAT1 inhibitor IA described in Ref. (16), 75 μM DGAT1 inhibitor IB (Tocris Bioscience). NT, not transfected. B, shown are Western blots analysis of cell extracts (left panels) or isolated LD fractions (right panels) from shRNA-expressing Huh7-Lunet cells. C, shown is real-time RT-PCR analysis of DGAT1 or DGAT2 mRNA expression levels in Huh7-Lunet cells transduced with the corresponding shRNAs (mean ± S.E.; n = 3 independent experiments).
NS5A to LDs and suppresses HCV particle production despite the continued presence of core at LDs (Fig. 8). It is not yet clear why the mutant DGAT1 does not misroute core as both proteins, core and NS5A, bind mutant DGAT1 effectively. Different membrane anchors of the two viral factors might be responsible for their distinct behaviors; although core is anchored to the ER membrane via a C-terminal transmembrane anchor that is processed by an intra-membranous signal peptide peptidase (27), NS5A associates post-translationally with the membrane surface via an N-terminal amphipathic α-helix (28) and is firmly anchored in the membrane. Future experiments will address whether this differential membrane association explains why NS5A is localized away from LDs by mutant DGAT1 and core localization is less affected or whether other factors play a role.

It remains to be discovered why DGAT1, and not DGAT2, is important for localizing core and NS5A proteins to LDs. The two DGAT enzymes, despite both producing triglycerides, are not part of the same family of proteins and have unrelated DNA sequences and protein structures. DGAT1 is predicted to contain multiple transmembrane domains and forms homotetramers (29). DGAT2 is less hydrophobic and possesses only one or two membrane spanning domains that allow DGAT2 itself to translocate to LDs, a feature not observed for DGAT1 (30).

DGAT1 has a dual membrane topology that exerts overt (cytosolic) and latent (ER luminal) DGAT activities (31). Wurie et al. (31) used two different DGAT1 inhibitors (including iA used in this study) with differential effects on overt and latent DGAT1 activities. As iA only inhibits overt DGAT1 activity at high concentrations and high concentrations of the drug are required to inhibit HCV infection and localization of HCV proteins to LDs (11), HCV may have evolved to specifically exploit overt DGAT1 activity in cells. This is consistent with a model that the activity serves to direct HCV core and NS5A to LDs. However, the two DGAT1 functions might not be completely distinct, and HCV infectious particle production in cells might be initiated in close contact with a triglyceride pool relevant for intraluminal VLDL secretion. Further studies of the mecha-
DGAT1 Localizes the HCV NS5A Protein to Lipid Droplets

DGAT1 inhibitors suppress NS5A localization to LDs reveal a drug discovery in HCV infection (33, 34), our findings that suppress HCV particle production without compromising diseases (32), may represent unique LD-targeting therapeutics, some in clinical trials for treating obesity-associated hepatoma cells treated with DGAT1 inhibitors (11), these triglyceride-synthesizing activity in these cells. Because its role in HCV infection.

mism of action of DGAT1 in LD biogenesis are required to clarify its role in HCV infection.

Our results provide evidence that LD association of HCV proteins is not a random process but requires active DGAT1-mediated LD formation. Strikingly, inhibition of DGAT1 activity in hepatoma cells does not decrease overall LD content, indicating that DGAT2 can compensate for the loss of DGAT1 triglyceride-synthesizing activity in these cells. Because DGAT2 activity is sufficient to maintain steady LD levels in hepatoma cells treated with DGAT1 inhibitors (11), these inhibitors, some in clinical trials for treating obesity-associated diseases (32), may represent unique LD-targeting therapeutics that suppress HCV particle production without compromising overall lipid metabolism. As NS5A itself has become a focus of drug discovery in HCV infection (33, 34), our findings that DGAT1 inhibitors suppress NS5A localization to LDs reveal a novel mechanism to explain how DGAT1 inhibitors interfere with HCV infection.

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DGAT1 Localizes the HCV NS5A Protein to Lipid Droplets
Diacylglycerol Acyltransferase-1 Localizes Hepatitis C Virus NS5A Protein to Lipid Droplets and Enhances NS5A Interaction with the Viral Capsid Core
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