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Identification and Comparative Analysis of Hepatitis C Virus-Host Cell Protein Interactions

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Abstract

Hepatitis C virus (HCV) alters the global behavior of the host cell to create an environment conducive to its own replication, but much remains unknown about how HCV proteins elicit these changes. Thus, a better understanding of the interface between the virus and host cell is required. Here we report the results of a large-scale yeast two-hybrid screen to identify protein-protein interactions between HCV genotype 2a (strain JFH1) and cellular factors. Our study identified 112 unique interactions between 7 HCV and 94 human proteins, over 40% of which have been linked to HCV infection by other studies. These interactions develop a more complete picture of HCV infection, providing insight into HCV manipulation of pathways, such as lipid and cholesterol metabolism, that were previously linked to HCV infection and implicating novel targets within microtubule-organizing centers, the complement system and cell cycle regulatory machinery. In an effort to understand the relationship between HCV and related viruses, we compared the HCV 2a interactome to those of other HCV genotypes and to the related dengue virus. Greater overlap was observed between HCV and dengue virus targets than between HCV genotypes, demonstrating the value of parallel screening approaches when comparing virus-host cell interactomes. Using siRNAs to inhibit expression of cellular proteins, we found that five of the ten shared targets tested (CUL7, PCM1, RILPL2, RNASET2, and TCF7L2) were required for replication of both HCV and dengue virus. These shared interactions provide insight into common features of the viral life cycles of the family *Flaviviridae*.

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Introduction

Hepatitis C Virus (HCV), like all viruses, relies on the host cell to provide functions not encoded within its own genome. During infection, the virus must interface with cellular pathways to create an environment conducive to viral replication. These changes in the infected cell are often mediated by direct physical interactions between viral and host proteins. Similarly, the host cell has evolved mechanisms to impede viral replication through direct interaction with viral factors. Identification of the interactions between virus and cellular proteins is, therefore, essential to understanding the complex interplay between HCV and its host. Likewise, comparing the virus-host cell interactions can reveal relationships between the viruses and provide insights into potentially important similarities and differences in virus biology. Previous studies have compared the cellular targets[1-5] and intraviral interactions[6, 7] of orthologous viral proteins across viral species. However, comparison of interactomes across more divergent viral species remains problematic due to difficulties in distinguishing orthology relationships between viral proteins.

HCV is a major burden on global health, with an estimated 170 million people infected worldwide.[8] Chronic HCV infection is associated with progression of several diseases, including liver cirrhosis, steatosis and hepatocellular carcinoma.[9] HCV pathology, persistence, and response to treatment are influenced by the genotype (GT) of the infecting virus.[10-12] These differences in biology suggest differences in the host cell interactions for the various GTs. However, systematic screens for interactions with host proteins have been performed only for HCV GT 1a and 1b proteins.[13, 14] The HCVPro database currently lists 104 and 359 cellular targets for GTs 1a and 1b, respectively, but only 9 cellular targets for GT 2a.[15] As a commonly studied laboratory strain, identification of host factors involved in the replication of HCV 2a will better inform future experiments with HCV culture systems.

HCV belongs to the family *Flaviviridae* and is the prototypical member of the genus *Hepacivirus*. Its positive-sense RNA genome encodes a polyprotein of approximately 3000 amino acids that is co-translationally processed into 11 mature peptides including three structural proteins: core, E1 and E2; 6 non-structural (NS) proteins: NS2, NS3, NS4A, NS4B, NS5A, NS5B; a putative viroporin, p7; and frameshift protein, F.[10] This small collection of gene products elicits the complex network of concerted changes in metabolic and regulatory processes that transform the phenotype of the host cell. Although the genomic organization is generally conserved among the *Flaviviridae*, HCV differs from most other family members in its mode of transmission and its capacity to establish persistent infection. While most of the members are transmitted via arthropod vectors, HCV is primarily transmitted through blood-blood contact.[8] Similarly, whereas *Flaviviridae* such as dengue virus (DENV) can establish acute hepatotropic infections that lead to hepatitis in some infected patients[16], HCV establishes chronic liver infections in about 70-80% of

infected individuals[8]. Comparisons of the cellular proteins targeted by the *Flaviviridae* are likely to provide insight into the differences in their replication and pathology.

Here we report the results of a large-scale yeast two-hybrid (Y2H) screen to identify virus-host cell protein interactions for the HCV 2a strain, JFH-1. The interactions identified augment the current understanding of HCV-host interactions and offer insight into HCV biology. We use the genome-wide collection of HCV 2a – host interactions identified in this study as the basis to compare interactomes of other HCV genotypes and other *Flaviviridae*.[17] Our results indicate that these comparisons are heavily influenced by experimental conditions and therefore, meaningful comparison of genome-wide interactomes requires parallel screening to reduce confounding inter-experimental variation. Comparing the HCV and DENV interactomes that were generated from parallel screens demonstrated that genome-wide comparisons can capture important biological similarities and differences between the two viruses. By probing a subset of the shared targets of HCV and DENV through siRNA knockdown, we identified host factors that were required for infection of both viruses, which may prove to be promising therapeutic targets.

Results

Mapping the HCV-Human protein interaction network

To identify human proteins targeted by HCV, we performed a genome-wide Y2H screen with 20 constructs encoding full-length genes or gene fragments representing 8 of the 11 HCV proteins (Fig. 1 and SI Table 1). Although screens to identify HCV-human protein-protein interactions have been reported, our screen differs in two key ways. First, while previous screens have focused on HCV GT 1b[13] or the NS4B and Core proteins from GT 1a[14], we generated our Y2H bait proteins from the JFH1 strain of HCV GT 2a. JFH1 was the first HCV strain to be successfully propagated in cell culture.[18-20] Since JFH1 and its derivatives are commonly used in the laboratory to study HCV replication, the interactions reported here will better inform future studies with this strain. Furthermore, HCV GTs vary in their pathology and their responsiveness to treatment; a comprehensive analysis of GT-specific virus-host cell interactions is expected to yield insight into the mechanistic basis for these differences. A second key difference in this study is that we employed a Y2H library derived from human liver RNA. In contrast, the previous genome-wide Y2H screen with GT 1b used cDNA libraries derived from human fetal brain and spleen.[13] However, liver is the major tissue targeted by HCV and, as such, our screen develops a more relevant picture of HCV-human protein interactions.

This study identified 112 interactions between 94 human proteins and 7 of the 8 HCV proteins investigated in this study (Fig. 2A and SI Table 2). Seventy-eight human proteins (83%) interacted with a single viral protein, while 14 (15%) interacted with 2 viral proteins and 2 (2%) interacted with 3 viral proteins. A subset of the targets of NS5B and NS3 were chosen for confirmation in independent, orthogonal assays. Of the 8 NS5B targets tested, 6 were confirmed by pull-down assay (Fig. 3A and B) and 7 were confirmed by split-luciferase assay (Fig. 3C). Of the 12 NS3 partners tested, 6 were confirmed by split-luciferase assay (Fig. 3D). This confirmation rate, 13 of 20, or 65%, is comparable to confirmation rates reported for other high quality large-scale Y2H screens.[21-23]

High-throughput 'omics' studies of infected cells have established that HCV infection leads to major changes in the transcriptome, proteome, and lipidome of the host cell. These data sets provide an independent benchmark by which to judge the effectiveness of our screen. To that end, we compared the cellular proteins identified in this screen to those from other large-scale studies on HCV-host cell interactions, including gene expression[24-26], proteomics[25, 27], siRNA[28-31], and protein-protein interactions[13, 14] (Fig. 2B-E and SI Table 3). Of the proteins identified in interactions with HCV, 45% were previously linked to HCV infection in at least one other screen and 17% were found in two or more (Fig. 2A). Similar levels of corroboration were found with each data source, consistent with the suggestion that each data set is incomplete and that a more comprehensive view of HCV infection can be obtained through the integration of data from diverse sources.

Features of the HCV interactome

The complete set of human proteins that interacted with HCV 2a proteins in this study was analyzed for enrichment in annotation terms using the DAVID Bioinformatics database[32] and GSEA[33] (SI Table 4 and SI Fig. 1). Enriched gene ontology (GO) terms with a Benjamini-Hochberg-adjusted[34] P-Value <0.05 are shown in Fig. 4A. To identify cellular functions targeted by HCV GT 2a, the enriched terms from the DAVID and GSEA analyses were subjected to hierarchical clustering and overlaid onto the HCV 2a-human interactome (Fig. 4B and SI Fig. 2). This analysis highlighted cellular processes known to be involved in HCV infection, such as lipid transport, and implicated new cellular functions, including the microtubule-organizing center (Fig. 4 and 5A). Other notable cellular targets with functions consistent with the known biology of HCV include regulators of cell metabolism and proliferation[35] (Fig. 5B), complement activation and coagulation[36] (Fig. 5C), and lipid and cholesterol trafficking and metabolism[37] (Fig. 5D). A detailed discussion of these cellular functions can be found in supplemental information (SI Discussion).

Comparative analysis of HCV GT 2a interactome

Comparisons of viral interactomes can highlight similarities and differences in replication strategy, host cell perturbation and disease progression associated with the individual viruses.[1, 38, 39] By increasing the number of host proteins reported to bind to HCV 2a proteins from 9 to 103, this study enabled preliminary comparisons of the cellular targets of HCV GTs 1a, 1b, and 2a (Fig. 6 and SI Table 5). For this analysis, the complete sets of cellular interacting proteins for HCV GTs 1a, 1b, and 2a were downloaded from the HCVpro database; HCV GT 2a-interacting proteins from HCVpro were combined with the interactions identified in this study. The HCV GT interactomes were then examined to determine the overlap of cellular interacting proteins and enriched annotation terms. More cellular proteins were shared between the interactomes of GT 1a and 1b than with that of 2a; no cellular proteins interacted with viral proteins from all three GTs. Similar results were obtained when enriched annotation terms from each data set were compared. HCV 1a and 1b were again more similar to each other than to HCV 2a, and only eight enriched terms were found in common. Although these results are consistent with the closer genetic relationship between GT 1a and 1b, the comparison is likely influenced by the incomplete coverage of each study and the different experimental techniques that were used.

In contrast to the comparison of HCV GT interactions, we observed greater overlap between the HCV 2a interactome reported here and proteins that interacted with DENV serotype 2.[17] These interactions were identified in a parallel screening approach, shown graphically in Fig. 1B, in which HCV GT 2a and DENV proteins were screened against the same human liver cDNA library. All unique human gene fragments from both screens were then recloned and tested for interactions with all HCV 2a and DENV clones in an array format. In our studies, HCV 2a and DENV shared 38 cellular targets, which represent 40% of the cellular proteins found to interact with HCV 2a (Fig. 7A and SI Fig. 3). The overlap between our DENV2 and HCV 2a interactomes is greater than between any of the interaction datasets for individual HCV genotypes (Fig. 6B). It is also greater than the overlap between our DENV interactome and interaction datasets for any HCV genotype from the HCVpro database (Fig. 6D). Thus, by using the same conditions and the same human constructs, we reduced variation between experiments that can lead to false-negatives and enabled a more meaningful comparison between the interaction networks.

Shared targets of HCV and DENV are required for virus replication

To assess the features of the shared and unique targets of HCV 2a and DENV from our parallel screens, we employed the DAVID database to identify enriched terms (Fig. 7 and SI Fig. 4, and SI Table 6). Terms enriched among the common targets of HCV and DENV included Wnt signaling, cell division and DEAD-box helicases (Fig. 7B). Comparison of the functional profiles of each virus revealed common enrichment of centrosomes and microtubule organizing centers, cytoskeleton, and chromosomal segregation. It also emphasized the extensive targeting of cellular lipid processes by HCV but not DENV (Fig. 7C). Terms enriched among the unique targets of HCV (56 proteins) and DENV (63 proteins) further highlighted differential targeting of lipid and cholesterol processes by HCV and suggests that DENV may interface with the cytoskeleton through a distinct set of proteins.

To determine if the common cellular targets play a role in the replication of HCV and/or DENV replication, we used RNA interference to inhibit expression of ten cellular proteins. Effects on virus replication were measured relative to cells treated with a negative control siRNA (Fig. 8). Six of the proteins were required for HCV production, whereas seven were required for production of DENV. The decrease in virus replication was not due to loss of cell viability (SI Fig 5). Inhibiting expression of five proteins (CUL7, PCM1, RILPL2, RNASET2, or TCF7L2) reduced replication of both viruses. Inhibition of SDCCAG8 specifically reduced HCV production while inhibition of KTN1 and CTNNB1 only affected DENV production. The percentage of virus-interacting proteins that were implicated in virus replication compares favorably to similar studies. For example, in an earlier Y2H screen conducted with influenza (INFV), of 95 human proteins identified, only 3 were required for replication and 8 were antagonistic.[40] These data suggest that the shared interactions represent biologically relevant targeting of the same cellular proteins by HCV and DENV.

Discussion

Here we report the first genome-wide screen for HCV GT 2a-human protein interactions. Our study identified 112 interactions between 7 HCV 2a proteins and 94 human proteins, which expands the list of host targets of HCV 2a by a factor of ten. Several observations indicate that the interactions are relevant to HCV infection and associated disease progression. First, a high percentage of the Y2H interactions can be confirmed by alternative approaches. Second, we observed extensive overlap with orthogonal studies of HCV-host cell interactions. Third, the host factors identified in this study were enriched in functions and cellular localizations consistent with the known biology of HCV, such as intracellular trafficking, lipid and cholesterol metabolism, cell survival and innate immunity. Finally, using siRNA experiments to inhibit expression of cellular proteins that interacted with HCV 2a, we found that 60% of the proteins tested were required for HCV replication.

Comparisons of the HCV- and DENV-host cell interactomes

The large-scale identification of virus-host cell interactions has enabled viruses to be compared and classified based upon their host targets.[1-3] However, the optimal strategies for performing such comparisons are still being developed.[1-3] In a study of the oncogenic HPV E6 and E7 proteins from 11 HPV GTs, a parallel screening approach was used to examine interactions with human proteins.[3] Differences in human binding partners of E6 and E7 from the different genotypes were used to develop interaction profiles that correlated with the genetic relationships between the viruses and that reflected phenotypic differences in pathology, tissue tropism and response to treatment.[3] In contrast, an analysis of flavivirus-host cell interactions disregarded differences in the binding partners and instead built a single, genus-level, consensus interactome that combined the virus-host interactions of the NS3 and NS5 proteins from six flaviviruses.[1] Although this approach minimized differences between flavivirus species, it had the benefit of creating a functional profile of NS3 and NS5 targets enriched in cellular processes that are likely to play a role in flaviviral infection.

While these studies demonstrated the utility of the alternative comparative approaches for virus-host interaction data, they used cellular targets of specific, orthologous viral proteins as the basis for the comparisons. However, comparing orthologous viral proteins is not always possible when considering more distantly related viral species. Such is the case with HCV and DENV, which have similar genomic organization but diverge in both the sequence and functions of their viral proteins. Furthermore, comparing the interaction partners of single viral proteins discounts the potential for viruses to target the same cellular protein with multiple viral proteins or with different viral proteins in different strains.

To overcome these limitations, we tested all HCV and DENV constructs against all human gene fragments identified in library screens with either virus and then compared the genome-wide interaction profiles from viruses. We found greater overlap among the targets of HCV and DENV than was observed for either dataset when compared to previously reported interactions for the same viruses.[13, 14, 41-43] Because stringent criteria were used to identify positives and the most promiscuous interacting proteins were removed from the dataset, the overlap between HCV and DENV was unlikely to be due solely to false-positives. Consistent with this interpretation, a high rate of validation was observed in both

orthogonal protein interaction assays and siRNA experiments (Fig. 3 and 8). Thus, the use of parallel screening and genome-wide interaction profiles yielded a more complete picture of the relationship between viruses.

Comparing the DENV and HCV interactomes revealed differences in terms enriched among the host targets that may reflect important differences in life cycle of these viruses. Lipid- and cholesterol-related terms were the most prominent features of the HCV network, whereas the DENV interactome showed far fewer interactions with proteins involved in these processes. This difference reflects the intimate dependence of HCV on lipid processes that extends beyond formation of replication complexes where modulation of lipid metabolism and trafficking are thought to be perturbed during DENV infection.[44-48] HCV proteins involved in replication complex formation, as well as structural proteins, interacted with components of the lipid and cholesterol pathways, suggesting roles for these processes in multiple stages of the viral life cycle. Differences were also noted in the mechanisms by which the two viruses targeted the cytoskeleton.

The 38 shared targets of HCV and DENV include a high percentage of proteins that were required for optimal virus replication. Six of the ten human proteins targeted by siRNA screens were required for HCV infection, while seven were required for DENV replication. Surprisingly, of the five proteins required for replication in both viruses, only two interacted with viral proteins with homologous functions: CUL7, a member of the E3 ubiquitin ligase complex, interacted with the RNA polymerase of both viruses, and TCF7L2, a transcription factor involved in Wnt signaling, associated with the NS3 serine proteases from both viruses. This observation suggests that interactions with cellular factors may not be restricted to viral proteins with homologous functions.

Microtubule organizing centers are a common target of HCV and DENV

The MTOC is emerging as a key point by which viruses access the cellular trafficking machinery (reviewed in [49]). Diverse viruses, including herpes simplex[50], adenovirus[51], influenza A[52, 53], mouse norovirus 1[54], and HIV[55] target the MTOC. Our observation that MTOCs were highly represented among the cellular proteins identified in our screen is consistent with a role for MTOCs in HCV replication (Fig. 4A). Enrichment of MTOC components was also observed in an earlier study of HCV GT 1-human PPIs[13] and among proteins that change in abundance in HCV-infected cells.[27] Both lipid droplets (LDs)[56] and viral replication complex (RC) components[57] traffic towards the perinuclear MTOCs during HCV infection. Silencing PCM1, which binds to and recruits other proteins to the MTOC[58], significantly reduced both HCV and DENV production (Fig. 8). We have previously shown that MTOC components were enriched among the cellular proteins targeted by DENV and that DENV NS5 colocalized with PCNT, a component of MTOC complexes that interacts with PCM1.[17] However, while both viruses target MTOCs, the differential effects of silencing of SDCCAG8 expression on HCV and DENV production suggests that these structures may play varying roles in *Flaviviridae* replication (Fig. 8). Further interrogation of these targets is necessary to elucidate the dependence of these viruses on cytoskeletal dynamics and cell cycle regulation.

Comparisons of the HCV GT-host cell interactomes

In contrast to the HCV and DENV interactomes, little overlap was observed between the interactomes of HCV GTs 1a, 1b, and 2a (Fig. 6). Although the HCV GTs exhibit phenotypic differences that stem from differences in their interactions with the host, the lack of overlap between the GT interactomes most likely represents limitations of the current datasets. The human interaction partners of the HCV GTs were derived from multiple experimental platforms[15], and as a consequence, the comparisons are likely influenced by the false negatives and intrinsic biases of each experimental approach. At this point, differences in the cellular proteins targeted and the enriched terms of the individual HCV GTs are best viewed as a reflection of the different aspects of HCV biology, as opposed to GT-specific host cell interactions. This study, in particular, revealed extensive targeting of cellular proteins involved in cholesterol and lipid metabolism that were missed in previous screens. A more thorough examination of the similarities and differences in the cellular proteins targeted by the HCV GTs will require parallel screening approaches similar to those we employed to compare the interactomes of DENV and HCV.

Overlap with large-scale HCV-host cell interaction studies

Although the majority of the interactions identified here are novel, 42 of the interacting proteins (45%) were implicated in HCV infection in at least one other independent data set. Surprisingly, the greatest overlap (23 proteins) occurred with changes in the proteome and transcriptome of infected cells[24-27], rather than with genome-wide siRNA screens (8 proteins)[31, 59, 60] or other HCV-human protein interaction datasets (18 proteins).[13, 14] Five proteins identified in this study were implicated in HCV infection in four or more independent screens. Since the studies used diverse methods, it is unlikely that these proteins represent common false positives. However, despite the multiple lines of evidence implicating them in HCV infection, the precise roles of these proteins in HCV are replication or pathogenesis not understood.

The two most frequently identified proteins among the data sets were apolipoprotein A1 (APOA1) and nucleobindin 1 (NUCB1). APOA1 is a key regulator of high-density lipoprotein formation and reverse cholesterol transport.[61] In our screen, APOA1 interacted with NS2, but has been reported to bind to HCV GT1a NS5A[62], HCV GT1b NS4B[14], and DENV2 NS2[17] in previous Y2H studies. APOA1 is significantly down-regulated in proteomic[27] and transcriptomic studies on infected cells[25, 26], and colocalizes with lipid droplets and NS5A in sub-genomic replicon-expressing cells.[62] Expression of APOA1 was significantly reduced in HCV-related tumors from clinical studies when compared to paired, non-tumorous tissues.[63] NUCB1 is a multifunctional protein that regulates cell signaling[64] and the unfolded protein response.[65] It interacted with NS5B in this screen, but was found to interact with HCV GT1a NS5A in an earlier study.[14] NUCB1 was up-regulated in proteomic studies of HCV infection in cell culture and was identified in the same study as a bottleneck in the host-virus protein interaction network.[27] Further study is needed to clarify the roles of these proteins in the HCV life cycle.

Conclusion

This study is the first systematic analysis of the cellular proteins targeted the HCV GT 2a strain JFH1. Since JFH1 and its derivatives are commonly used laboratory strains, the interactions reported here will facilitate future studies on HCV-host cell interactions. These interactions highlighted key features of the HCV 2a – host interactome, such as lipid and cholesterol trafficking and metabolism, but also identified many novel cellular targets, including components of the microtubule-organizing center and key regulators of cellular proliferation. This study also comprised the first demonstration of comparative interactomics that employs genome-wide interaction networks resulting from parallel Y2H screening as a basis for characterizing differences in viral life cycles and pathogenesis between HCV and DENV. This approach provided a greater overlap between identified partners than comparisons of datasets resulting from disparate screening procedures. Interrogation of cellular proteins that interacted with HCV and DENV implicated components of the centrosome/microtubule-organizing center, ubiquitinylation, RNA processing and lysosomal trafficking machinery of the host cell in the life cycles of both viruses. Taken together, these results suggest new avenues of study for HCV and demonstrate promising new comparative approaches to virus-host interactions that may lead to the development of broadly acting antivirals directed at shared host targets.

Methods

Additional experimental details can be found in Supplementary information.

Y2H screens

The overall strategy to identify human proteins that bind to HCV proteins is shown in Fig. 1. Briefly, HCV genes and gene fragments were cloned into the DNA-binding domain pOBD2 and screened at least twice against a human liver cDNA library as described in [17] and in supplemental information. To confirm the interactions, all unique human gene fragments from the initial library screens were recloned into the activation domain plasmid pOAD.103 and retested against all HCV constructs. In addition, all HCV constructs were tested for interactions with human proteins identified in a parallel screen with DENV proteins [17]. Y2H retests were performed in quadruplicate in a 384-spot array and monitored for growth on Y2H selection media lacking histidine or adenine; to be considered positive in the retest, at least three of the four replicates were required to demonstrate growth on both Y2H selection media.

The protein interactions from this publication have been submitted to the IntAct molecular interaction database [66] and assigned the identifier IM-21137.

Network generation and data mining

Analysis of the data set for enrichment in functional terms was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7[32] and Gene Set Enrichment Analysis (GSEA) v2.0.7.[33] To summarize the enriched annotation terms and associated proteins, the output from DAVID and GSEA were clustered and plotted using the

heatmap function in the R “stats” package. These clusters were used to generate Fig. 4B. Network diagrams were generated using Cytoscape v2.8.[67]

Validation of Y2H interactions

Split-luciferase assays were performed as described.[17, 68] Significance was determined by a one-tailed, unpaired Student's t-test. Co-purification experiments were performed using *E. coli*-expressed and purified maltose binding protein (MBP)-hexahistidine-tagged HCV proteins and *in vitro* translated HCV proteins. Additional details can be found in Supplemental Methods.

siRNA knockdown experiments

RNAi assays to assess the contribution of cellular proteins to HCV and DENV replication were performed as described in [46, 59] and in supplementary methods. Cell viability of siRNA-treated cells was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega) or the MTT assay. HCV or DENV production was compared to viral production in cells treated with a non-specific siRNA control. Significance was determined by one-tailed, unpaired t-test using the GraphPad Prism 5 software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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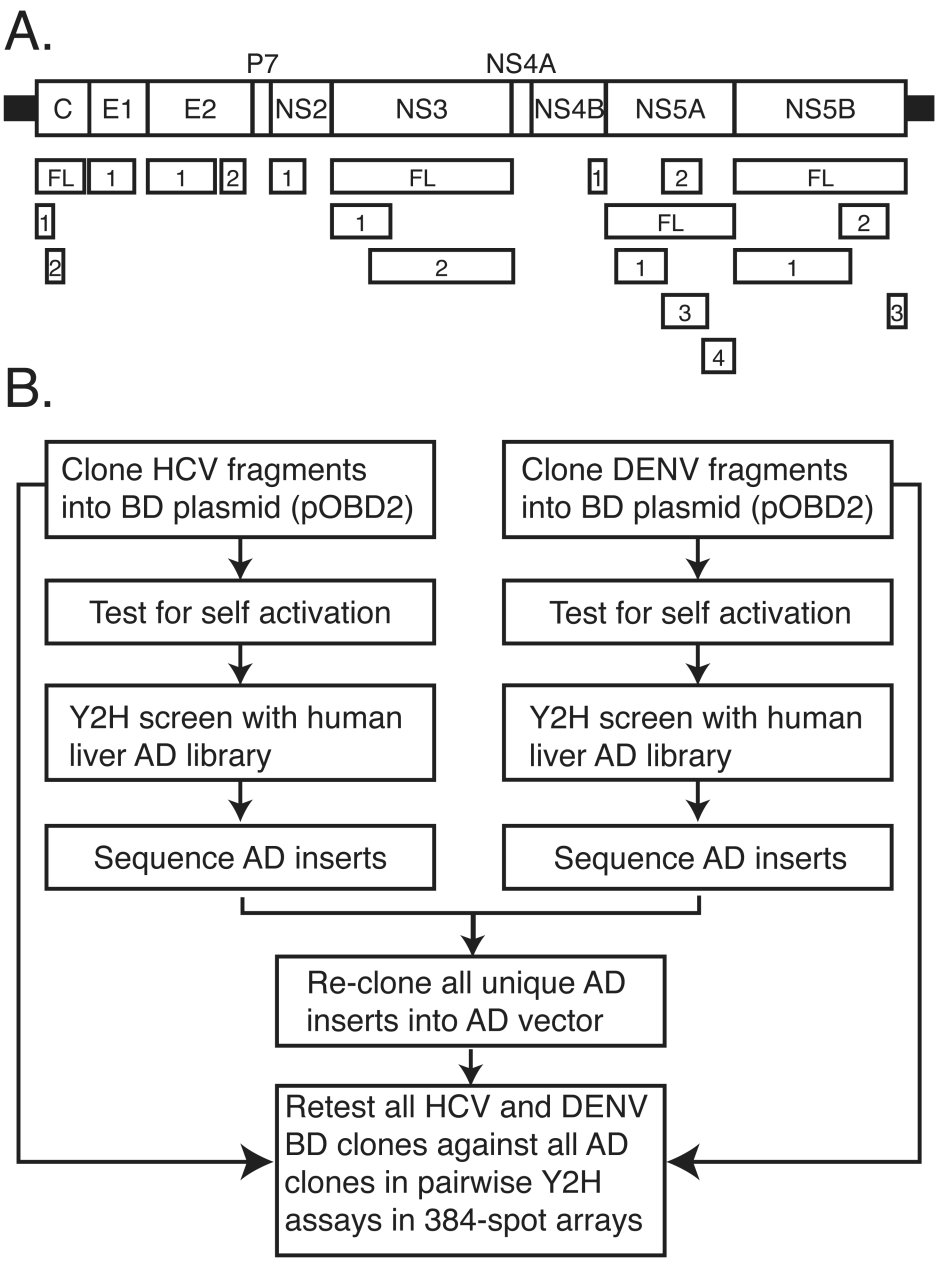

Fig. 1.

Overview of yeast two-hybrid (Y2H) screens to identify cellular proteins that bind to HCV proteins. (A) The organization of hepatitis C virus (HCV) genome and fragments used in Y2H screens of a human liver cDNA library. (B) Flowchart of the Y2H approach to screen for host proteins targeted by HCV and dengue virus (DENV).

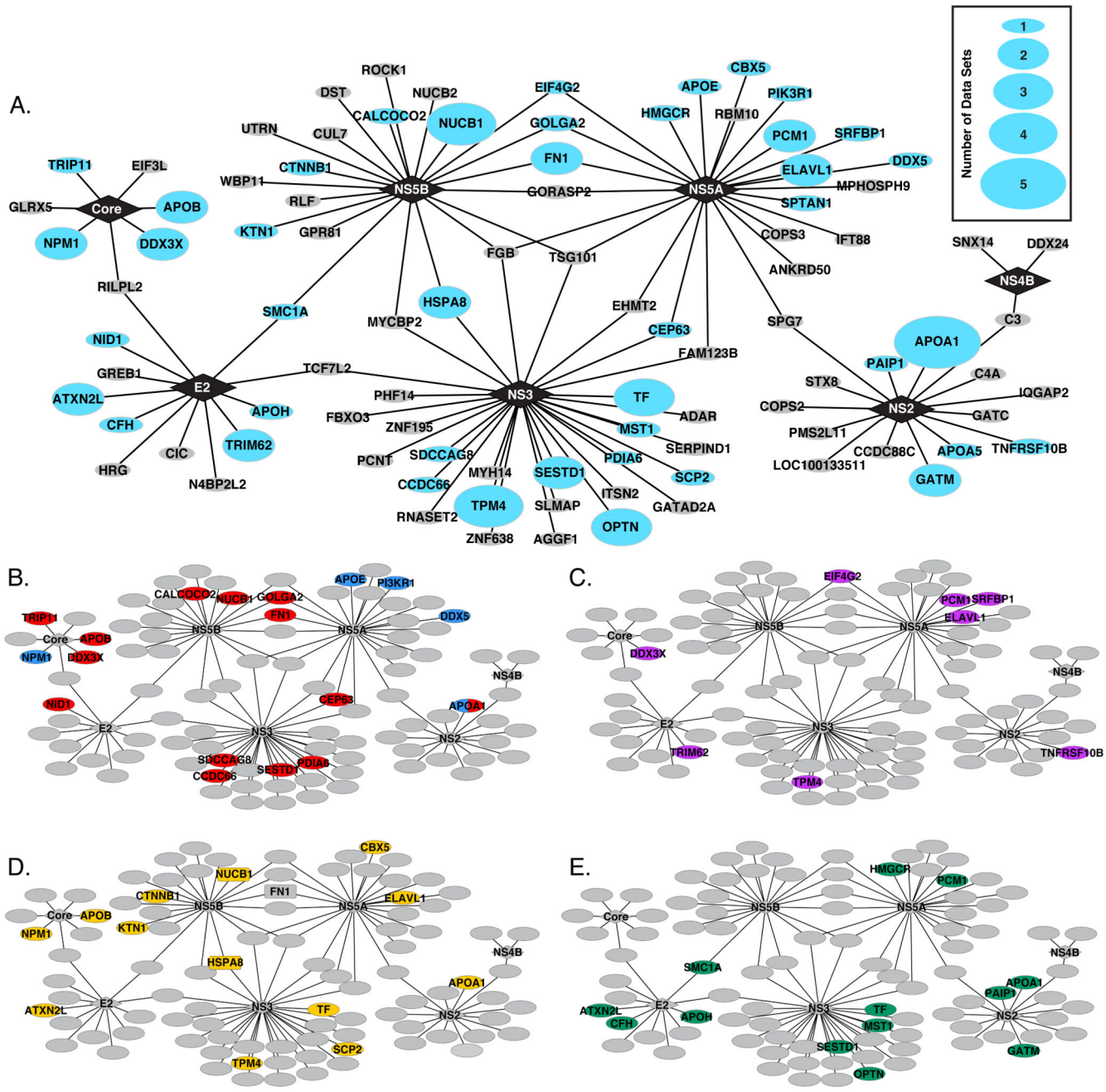

Fig. 2.

HCV 2a-human interaction network. (A) Interactions between HCV 2a and human proteins identified in this study. Diamonds indicate HCV proteins; ovals, human proteins; black lines, HCV 2a-human protein interactions. Proteins linked to HCV infection by other *in vitro* HCV-host cell interaction experiments are shown in green. Node size indicates the number of independent data sets linking the corresponding gene to HCV replication. (B-E) Human proteins previously implicated in HCV infection by experiment type. (B) Physical interactions identified by Y2H, red nodes; or other experimental methods, blue nodes; (C) siRNA screens, purple nodes; (D) proteomics, yellow nodes show differentially expressed

proteins; rectangles indicate bottleneck proteins as defined in [27]; (E) microarray, green nodes.

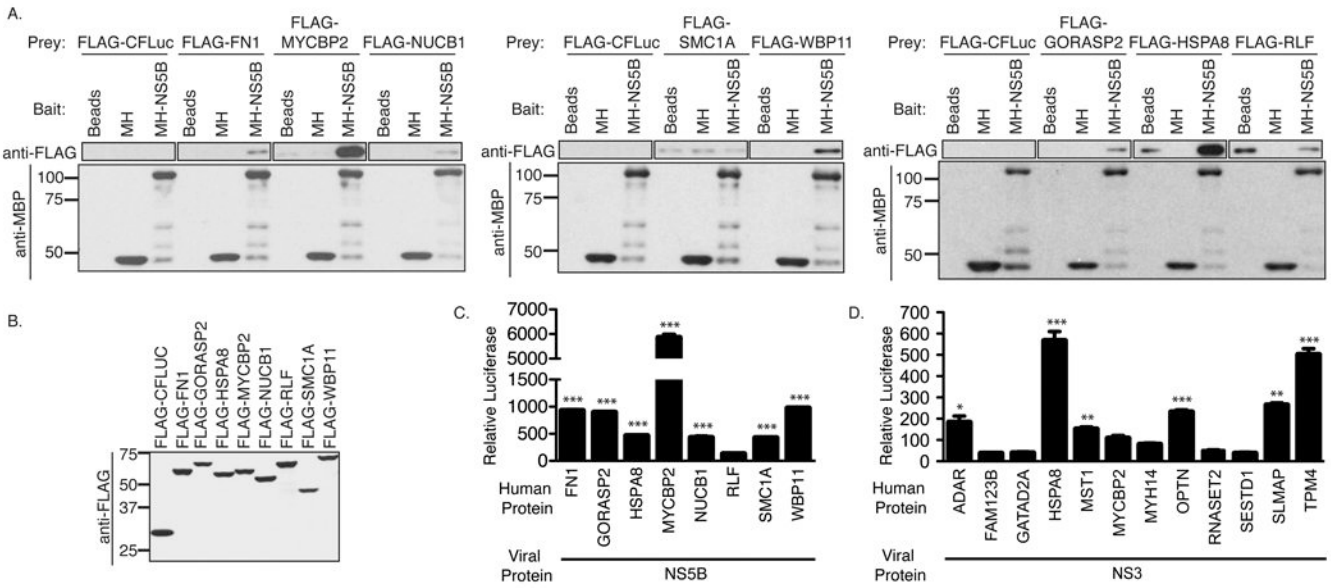

Fig. 3.

Confirmation of HCV 2a-human protein interactions. (A) Co-purification of HCV human proteins with NS5B. Maltose-binding protein-hexahistidine (MH) pull-down experiments to confirm NS5B-host interactions were performed as described in Experimental Procedures and Supplementary Methods. Purified complexes were analyzed by western blot probed with antibodies against the FLAG-epitope tag or maltose binding protein (anti-MBP). (B) Western blots of input prey protein from *in vitro* translation reactions for NS5B pull-down experiments. (C) Confirmation of NS5B- and NS3-host interactions in the split-luciferase assay. Relative luciferase activity was normalized to the N-FLuc negative control, which is shown as 100 RLU. *, **, and *** indicate P< .05, P<.005 and P < .0005, respectively.

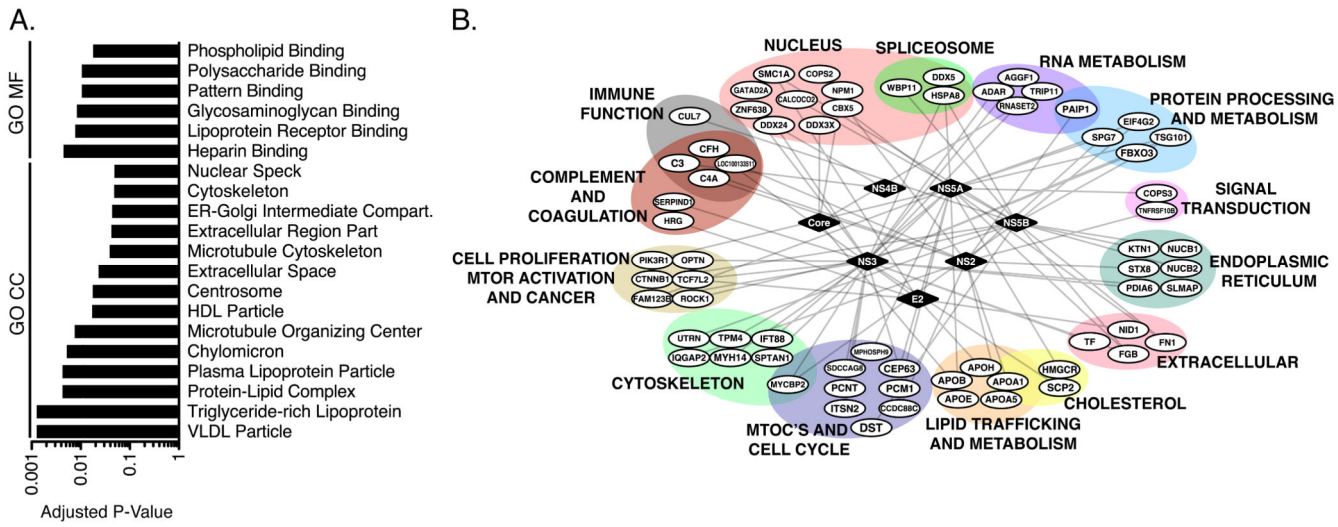

Fig. 4.

Enriched annotation terms in the cellular targets of HCV. (A) Gene Ontology cellular component (CC) and molecular function (MF) annotations enriched among the set of human proteins that interacted with HCV 2a identified using the DAVID Bioinformatics Database. Graph shows the $-log_{10}$-transformed Benjamini-corrected P-values for each term. Terms were considered significantly enriched if the Benjamini-adjusted P-value was less than 0.05. (B) Functional map of HCV targets. A subset of human proteins that interacted with HCV 2a grouped according to their cellular functions based on Gene Set Enrichment Analysis. Viral proteins, black diamonds; Human proteins, ovals; Virus-human interactions, black lines; Human-human interactions, red lines.

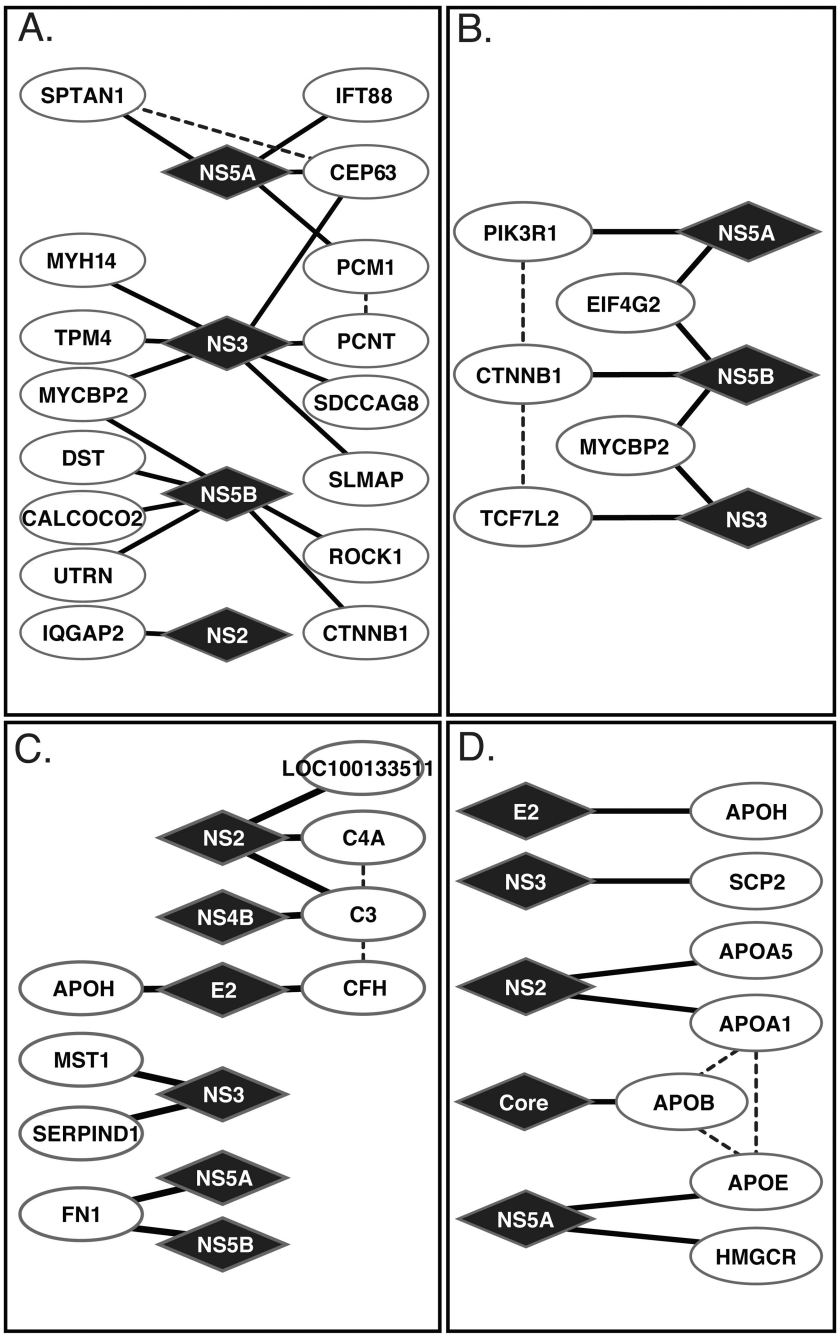

Fig. 5.

Cellular pathways targeted by HCV. Subnetworks highlighting the interactions with human proteins involved in (A) cytoskeletal (left) and microtubule-organizing center (right) targets, (B) cell proliferation and anti-apoptotic processes, (C) coagulation (left) and complement (right), and (D) lipid- and cholesterol-related functions. Viral proteins, black diamonds; human proteins, ovals; virus-human interactions, black lines; human-human interactions, dashed lines.

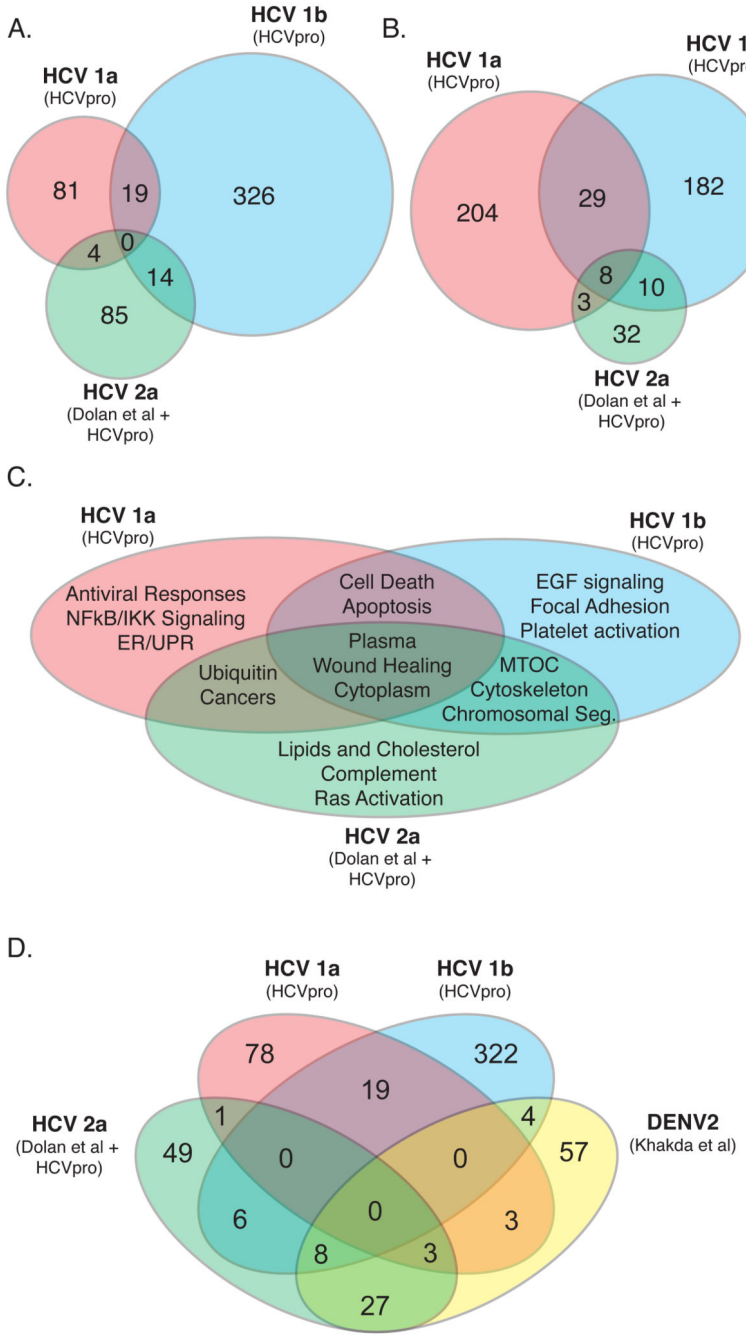

Fig. 6.

Comparison of the human proteins targeted by HCV genotypes. (A) The overlap of cellular proteins that interacted with HCV GTs 1a, 1b, and 2a. HCV-human protein interactions were identified in the current study (GT 2a) or were downloaded from the HCVpro database (GTs 1a, 1b, 2a). [15] References for all interactions from the HCVpro database are provided in Supplementary Information. (B) The overlap in enriched functional terms among the targets of each HCV GT. (C) Functions of cellular proteins that interacted each HCV GT. Additional details are provided in SI Table 3. (D). Comparison of cellular proteins that

interacted with individual HCV genotypes or DENV2. Cellular proteins that interacted with DENV2 were identified in a parallel with the screen for HCV GT 2a interactors. [17]

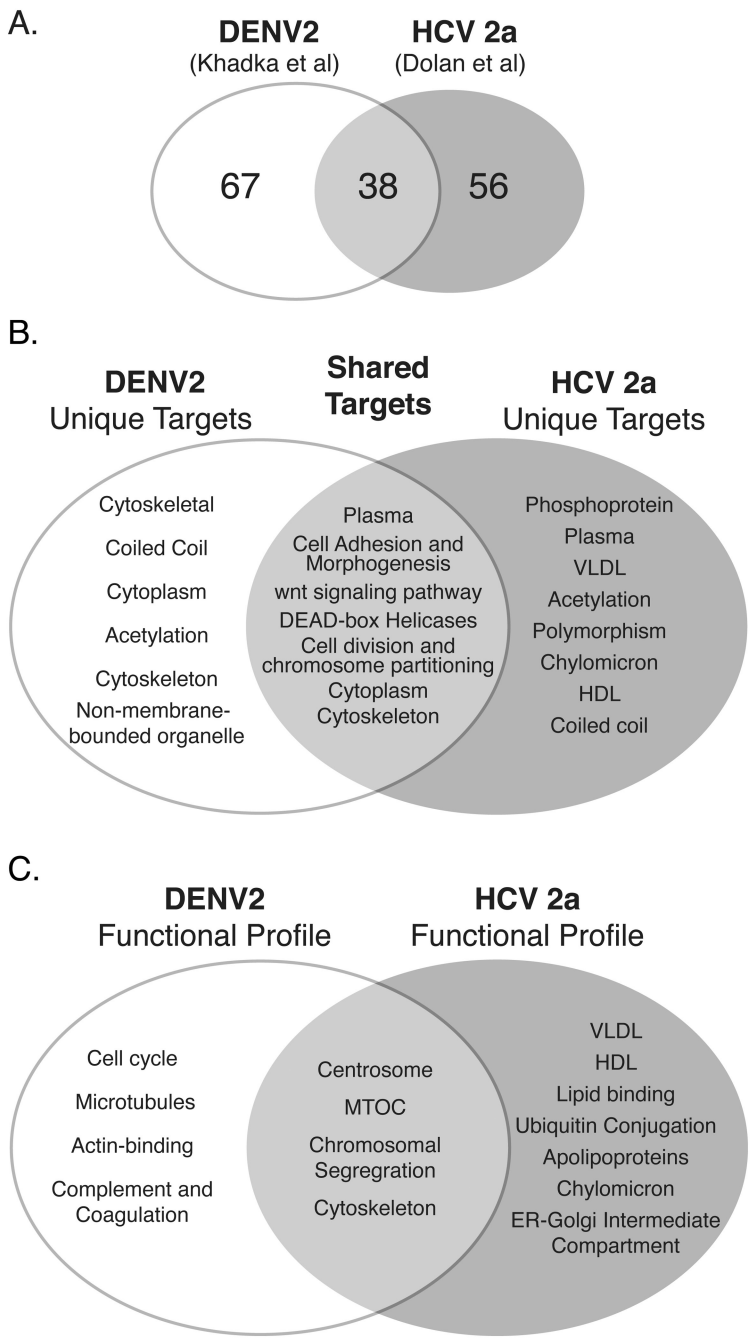

Fig. 7.

Cellular proteins targeted by HCV and DENV. (A) Venn diagram showing the overlap between cellular proteins that interacted with HCV GT 2a (this study) and DENV serotype 2 (Khadka et al.). [17] (B) Cellular functions associated with human proteins that interacted specifically with DENV2 or HCV GT 2a (“Unique”), or with both viruses (“Shared”). DENV2 and HCV GT 2a interactions were identified in parallel Y2H screens. [17] (C) Comparison of the enriched annotation terms (“Functional profiles”) of cellular proteins that

interacted with HCV 2a and DENV2. An outline of the data analysis workflow is presented in SI Fig. 4. The complete list of enriched terms is provided in SI Table 4.

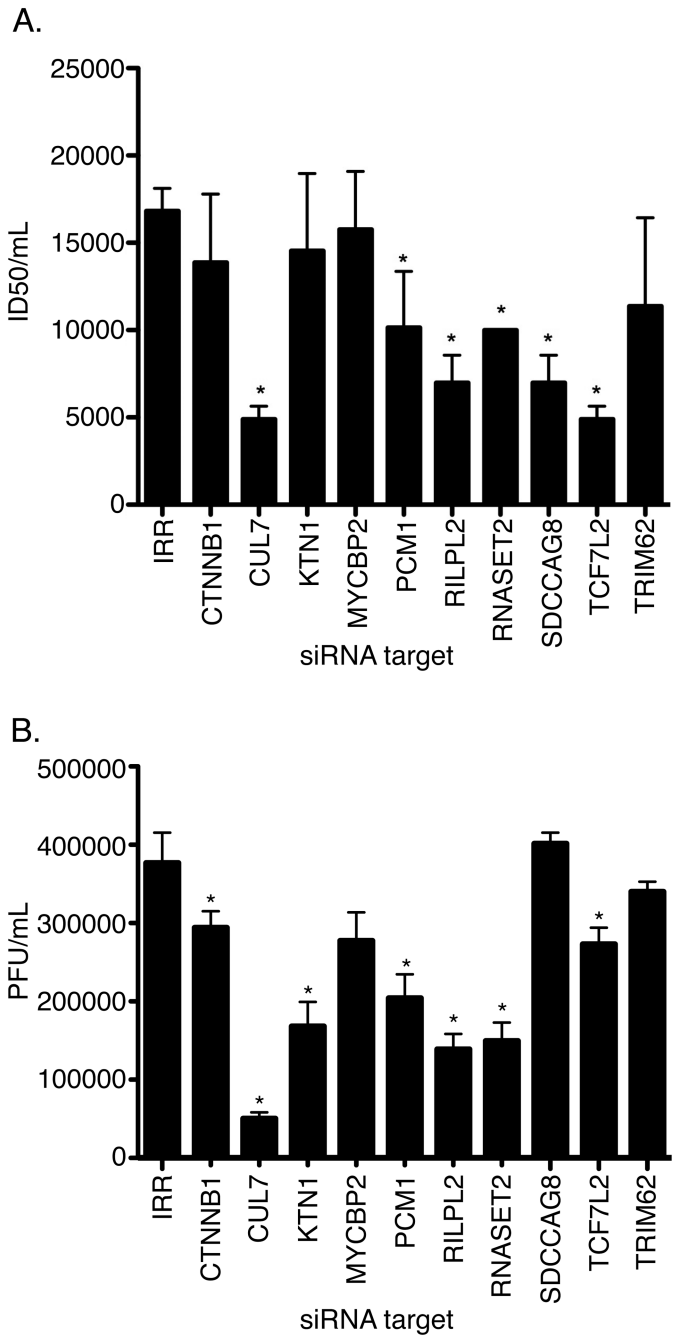

Fig. 8.

Common targets of HCV and DENV are required for replication. The requirement of shared cellular targets for the production of HCV and DENV was assessed using gene-specific siRNAs to reduce expression of the cellular proteins. (A) HCV production in Huh 7.5 cells was quantified by limiting dilution assay and (B) DENV production in Huh 7 cells was quantified by plaque assay. In both cases, virus production was compared to cells treated

with an irrelevant siRNA (IRR on graph), which is not homologous to any known human gene. * indicates $P < 0.05$ based on a one-tailed t-test.