SEC14L2 enables pan-genotype HCV replication in cell culture

Mohsan Saeed¹, Ursula Andreo¹, Hyo-Young Chung¹, Christine Espiritu¹, Andrea D. Branch², Jose M. Silva³ & Charles M. Rice¹

Since its discovery in 1989, efforts to grow clinical isolates of the hepatitis C virus (HCV) in cell culture have met with limited success. Only the JFH-1 isolate has the capacity to replicate efficiently in cultured hepatoma cells without cell culture-adaptive mutations¹–³. We hypothesized that cultured cells lack one or more factors required for the replication of clinical isolates. To identify the missing factors, we transduced Huh-7.5 human hepatoma cells with a pooled lentivirus-based human complementary DNA (cDNA) library, transfected the cells with HCV subgenomic replicons lacking adaptive mutations, and selected for stable replicon colonies. This led to the identification of a single cDNA, SEC14L2, that enabled RNA replication of diverse HCV genotypes in several hepatoma cell lines. This effect was dose-dependent, and required the continuous presence of SEC14L2. Full-length HCV genomes also replicated and produced low levels of infectious virus. Remarkably, SEC14L2-expressing Huh-7.5 cells also supported HCV replication following inoculation with patient sera.

Mechanistic studies suggest that SEC14L2 promotes HCV infection by enhancing vitamin E-mediated protection against lipid peroxidation. This provides a foundation for development of in vitro replication systems for all HCV isolates, creating a useful platform to dissect the mechanisms by which cell culture-adaptive mutations act.

Hepatitis C virus (HCV) is a leading cause of liver disease worldwide, with an estimated global burden of 185 million chronic infections⁴. Studies of this important human pathogen have historically been hampered by the lack of a cell culture system that supports replication of clinical isolates. Inoculation of primary human hepatocytes or hepatoma cell lines with serum from HCV-infected patients leads to extremely little, if any, replication. In addition, molecularly cloned HCV genomes that are infectious in experimentally infected chimpanzees fail to establish infection in Huh-7-derived human hepatoma cells⁵–⁸. Drug-selectable subgenomic replicons derived from these genomes must acquire mutations to replicate in cell culture⁹–¹².

Figure 1 | cDNA screening of Huh-7.5 cells identifies SEC14L2 as a critical host factor for HCV RNA replication. a, Illustration of the shRNA or cDNA screen. b, Three Huh-7.5 cell populations independently transduced with a pooled lentiviral cDNA library and a population of empty-vector-expressing control cells was electroporated with the indicated wild-type HCV subgenomic replicons (SG-neo) or a replication-defective S52 GNN replicon (3a pol–). After 3 weeks of G418 selection, the resulting cell colonies were stained with crystal violet. c, HCV NS3-NS5B region from 45 cell colonies was amplified by reverse transcription followed by PCR (RT–PCR) and subjected to direct sequencing. Shown are the per cent colonies harbouring wild-type, mutant, or a mixture of wild-type and mutant sequences. d, The SEC14L2 cDNA was amplified from 45 colonies (see Methods). CR, constant region; L, ladder; UC, untransduced cells; EV, empty-vector-transduced cells; NTC, no template control.

¹Center for the Study of Hepatitis C, Laboratory of Virology and Infectious Disease, The Rockefeller University, New York, New York 10065, USA. ²Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA. ³Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA.

©2015 Macmillan Publishers Limited. All rights reserved
This replication block could be due to the presence of inhibitory factor(s) that limit replication and/or the lack of essential host factor(s).

To overcome the block, we transduced Huh-7.5 cells with lentivirus libraries expressing either short hairpin RNAs (shRNAs) or complementary DNAs. The transduced cell populations were then electroporated with in vitro transcripts of wild-type G418-selectable genotype 3a and 4a HCV subgenomic replicons, which in unaltered Huh-7.5 cells require at least two adaptive mutations for replication (Fig. 1a). While the shRNA library did not yield any hits, the cDNA library produced numerous colonies (Fig. 1b). The vast majority of these colonies (34 of 45) harboured replicons with the parental sequence (Fig. 1c); mutations were present in the remaining colonies but none corresponded to known adaptive changes. To confirm their ability to support non-adapted replicons, cell colonies were treated with anti-HCV compounds to clear replicating viral genomes and then re-transfected with a second set of wild-type replicons. Selection with G418 resulted in the production of numerous G418-resistant colonies. In contrast, no colonies were produced from control cells cured of cell culture-adapted replicons (Extended Data Fig. 1).

While the shRNA library did not yield any hits, the cDNA library resulted in the production of numerous G418-resistant colonies. In contrast to wild-type HCV replicons, cell culture-adapted replicons showed a mild to moderate response to SEC14L2 expression (Extended Data Fig. 3c), indicating that cell culture-adaptive mutations partially circumvent the need for SEC14L2. This notion is further supported by the fact that wild-type replicons in the presence of SEC14L2 yielded almost as many colonies as were obtained for cell culture-adapted replicons in the absence of SEC14L2. Similarly, replication of the JFH-1 subgenomic replicon, which does not require adaptive mutations, was not enhanced by SEC14L2 (data not shown).

To examine replication of full-length HCV genomes, we took two approaches. First, we performed transient replication assays in Huh-7.5 cells stably expressing an HCV infection-dependent fluorescence relocalization (HDFR) reporter (Fig. 2a), with or without SEC14L2. HCV RNA replication in HDFR cells results in nuclear localization of otherwise mitochondrially anchored red fluorescent protein (RFP). When in vitro transcribed full-length HCV RNA genomes were transcribed in vitro.

**Figure 2 | SEC14L2 allows replication of wild-type HCV subgenomic replicons.** a. Cell colonies obtained for the indicated replicons in Huh-7.5 cells transduced with empty vector or SEC14L2. b. HCV RNA levels for wild-type replicons in SEC14L2-expressing cells (black bars) and two cell culture-adapted replicons in control cells (grey bars) are shown. c. Schematic representation of the doxycycline-inducible SEC14L2–enriched green fluorescent protein (EGFP) expression plasmid (top panel). Dose-dependent effect of doxycycline (Dox) on SEC14L2–EGFP expression (middle panel) and SEC14L2–EGFP replication (bottom panel) is shown in two single cell clones. EMCV IRES, encephalomyocarditis internal ribosomal entry site; CFU, colony-forming units per 100,000 transfected cells. rtTA3, reverse tetracycline-controlled transactivator 3. d. The colonies obtained for 1 μg ml⁻¹ doxycycline in c were pooled and passaged in G418-free medium in the presence or absence of doxycycline. After 2 weeks, SEC14L2 protein was undetectable in cells passaged without doxycycline (top panel). These cells died when subjected to G418 selection (bottom panel). Bar graphs show means ± s.d. from at least duplicate experiments.
electroporated into SEC14L2-expressing HDFR cells (SEC14L2/ 
HDFR), varying numbers of positive cells were visible after 6 
days (Extended Data Fig. 4a, b). These cells had readily detectable 
levels of HCV RNA that could be suppressed by treatment with 
daclatasvir, a specific antiviral targeting NS5A (Fig. 3a), confirming 
authentic HCV replication. As expected, no evidence of viral replication was 
seen in control HDFR cells.

For stable replication assays, we inserted a blasticidin S deaminase 
(BSD) cassette into full-length HCV genomes and introduced them 
to SEC14L2/Huh-7.5 cells by electroporation. Selection with blasti-
cidin resulted in large numbers of colonies that were positive for 
RNA and protein (Fig. 3b, c). Inoculation of naive SEC14L2/Huh-7.5 
cells with culture medium from these colonies and subsequent selec-
tion with blasticidin yielded varying numbers of colonies that were 
positive for viral RNA and protein, indicating production of infectious 
virus. Incubation of cells with anti-CD81 antibody before inoculation 
reduced colony formation (Extended Data Fig. 4c–e). Thus, SEC14L2 
positive for viral RNA and protein, indicating production of infectious 
replication with blasticidin yielded varying numbers of colonies that were 
in SEC14L2/Huh-7.5 cells transduced or not to express SEC14L2. 
Selection with blastidin resulted in large numbers of colonies that were 
positive for viral RNA and protein, indicating production of infectious 
virus. As expected, no evidence of viral replication was seen in control 
HDFR cells.

Past attempts to infect cultured cells with clinical HCV isolates have 
largely remained unsuccessful. Patient sera are infectious when inocu-
lated into chimpanzees or human-liver-chimaeric mice13,14, but do not 
result in productive infection in hepatoma cells. To test if SEC14L2 
facilitates infection with HCV from infected sera, we inoculated 
SEC14L2/HDFR cells with six high-titre HCV sera of genotypes 1a, 
1b and 3a. Large numbers of cells with nuclear RFP were observed 
for genotype 3a, followed by genotype 1b (Fig. 3d, bottom panel). 
All serum samples yielded detectable levels of HCV RNA that were 
sensitive to treatment with daclatasvir (Fig. 3d, top panel). In contrast, 
control cells did not support HCV RNA replication.

A number of experiments were undertaken to examine SEC14L2-
specific determinants important for its HCV replication enhancing 
effects. Cells expressing murine SEC14L2 were highly permissive to 
wild-type HCV (Extended Data Fig. 5a). Naturally occurring splice 
isosforms of SEC14L2, encoding shorter than the full-length proteins, 
did not facilitate HCV replication (Extended Data Fig. 5b, c). Two 
other SEC14L2-related proteins have been described in humans: 
SEC14L3 and SEC14L4 (ref. 11), none of which could be detected in 
Huh-7.5 cells (Extended Data Fig. 5d). While SEC14L3 allowed low 
level HCV replication, SEC14L4 had no effect (Extended Data Fig. 5d).

SEC14L2 has three domains: an amino-terminal CRAL-TRIO 
domain (amino acids 12–60) that binds small lipophilic molecules15, 
a SEC14-like domain (amino acids 76–245) that contains a hydro-
phobic ligand-binding pocket, and a carboxy-terminal GOLD domain 
(amino acids 300–380) that is postulated to facilitate protein–protein 
interactions. To test the importance of these domains in facilitating 
HCV replication, we constructed various deletion mutants (Extended 
Data Fig. 5e) and SEC14L2–SEC14L4 chimaeras (Extended Data 
Fig. 5g). Neither the deletion mutants nor any of the chimaeric con-
structs conferred HCV permissiveness to Huh-7.5 cells (Extended 
Data Fig. 5f, b), suggesting that multiple SEC14L2-specific determi-
nants are required for HCV replication.

In terms of mechanism, yeast two-hybrid, co-immunoprecipitation 
and subcellular fractionation did not reveal a physical interaction 
between SEC14L2 and HCV replicase components (Extended Data 
Fig. 6a–c). Since SEC14L2 is implicated in the regulation of the 

**Figure 3 | SEC14L2 allows replication of full-length HCV genomes.** a, HCV 
RNA levels 6 days after electroporation of the indicated full-length HCV 
genomes in HDFR reporter cells transduced with empty vector or SEC14L2. 
DMSO or daclatasvir (10 nM) was added 3 days before cell harvest. b, Cell 
colonies obtained for the indicated blasticidin-selectable, full-length (FL-BSD) 
HCV genomes in Huh-7.5 cells transduced or not to express SEC14L2. 
c, The cell colonies obtained in b were pooled and HCV RNA and protein 
abundance was determined by reverse transcription followed by quantitative 
PCR (qRT-PCR; top panel) and immunoblotting with anti-NS3 antibody 
(bottom panel). d, Direct infection of SEC14L2-expressing HDFR reporter 
cells with 6 serum samples from HCV patients. The top panel shows HCV 
RNA levels. The bottom panel shows the nuclear localization of RFP in 
in individual cells infected with serum 2 (genotype 1b) and serum 6 (genotype 3a). 
White arrows indicate cells with nuclear RFP. Bar graphs show means ± s.d. 
from at least duplicate experiments.
phosphoinositide 3-kinase (PI3K)/Akt signalling pathway, cholesterol synthesis, and vitamin E metabolism, we hypothesized that modulation of these pathways might be involved in facilitating HCV RNA replication. As previously reported for prostate cancer cells, SEC14L2 expression mildly reduced Akt phosphorylation in Huh-7.5 cells (Extended Data Fig. 7a); yet suppression of PI3K/Akt pathway by SEC14L2-independent methods failed to support HCV replication (Extended Data Fig. 7b–d).

SEC14L2 expression transiently stimulates cholesterol synthesis in rat hepatoma cells. However, we did not see increased cholesterol levels in Huh-7.5 cells stably expressing SEC14L2 (data not shown). Furthermore, a SEC14L2 mutant lacking cholesteroletic activity efficiently supported HCV replication (Extended Data Fig. 7e, f). In contrast, a SEC14L2 deletion mutant capable of stimulating cholesteroletic enzymes in rat hepatoma cells failed to confer HCV permisiveness to Huh-7.5 cells (1–383 mutant in Extended Data Fig. 5e, f). Taken together, SEC14L2 phenotype cannot be attributed to stimulation of the cholesterol synthesis pathway.

We next examined the role of SEC14L2 as a vitamin E (VE; tocopherol)-binding protein. Lipid-soluble antioxidants such as VE inhibit lipid peroxidation and enhance HCV replication. As described for prostate cancer cells, SEC14L2 enhanced the accumulation of VE in Huh-7.5 cells (Fig. 4a). Interestingly, HCV isolates resistant to lipid peroxidation (JFH-1, Jc1, H77D, TNcc) and not enhanced by VE were insensitive to SEC14L2 expression. In contrast, replication of a lipid peroxidation-sensitive clone, H77S.3 (ref. 20), was enhanced to similar levels by VE and SEC14L2 (Fig. 4b and Extended Data Fig. 8a, b). SEC14L2 and VE did not show an additive effect on H77S.3 replication (Fig. 4c, d), suggesting an overlapping mechanism. The replication enhancing effect of other lipophilic anti-oxidants was also markedly suppressed in the presence of SEC14L2 (Extended Data Fig. 8c–f).

SEC14L2 also reduced the inhibitory effect of lipophilic oxidants on HCV replication, but not that of interferon-alpha or cyclosporine A (Fig. 4e and Extended Data Fig. 8g–j), indicating selective protection against lipid peroxidation. Consistent with this hypothesis, SEC14L2 expression lowered the intracellular abundance of malondialdehyde, a secondary product of lipid peroxidation (Fig. 4f). Furthermore, SEC14L2 phenocopied the reported effects of other lipid peroxidation inhibitors such as VE and sphingosine kinase inhibitor by enhancing the 50% effective concentration (EC50) of NS3 protease (Danoprevir) and NS5B polymerase (2′CmeA) inhibitors against H77S.3, but not against lipid peroxidation-resistant Jc1 (Extended Data Fig. 8k–l).

SEC14L2 no longer supported HCV replication when cells were cultured in medium containing fetal bovine serum (FBS) that had been depleted of lipid-soluble materials including VE (delipidated FBS) (Fig. 4g, h), an effect that was reversed by VE supplementation (Fig. 4h), suggesting that the antioxidative effect of SEC14L2 is mediated, at least in part, through VE. Since delipidated FBS lacks many other lipids, we cannot entirely rule out the involvement of other potential SEC14L2 ligands in HCV replication. Recently, sphingosine kinase-2 (Sphk2) was also shown to promote lipid peroxidation and inhibit replication of non-JFH1 isolates. However, neither messenger RNA nor protein levels of Sphk2 were diminished by SEC14L2 expression. In addition, inhibition of Sphk2 with sphingosine kinase inhibitor or by stable expression of dominant-negative Sphk2(G212D) (ref. 21) did not allow HCV RNA replication in Huh-7.5 cells (data not shown), suggesting that the SEC14L2 effect is not mediated by inhibiting Sphk2.

Collectively, these data imply that SEC14L2 promotes HCV replication by enhancing VE-mediated inhibition of lipid peroxidation. As expected, RNA viruses insensitive to lipid peroxidation did not respond to SEC14L2 expression (Extended Data Fig. 9). Interestingly, although VE supplementation significantly enhanced SEC14L2-mediated replicon colony formation (Extended Data Fig. 10), it failed to support colony formation on its own (data not shown), suggesting that SEC14L2 might have additional functions. SEC14L2-mediated enrichment of VE at specific sites may be required to support stable HCV replication, but further studies will be required to elucidate the precise mechanism by which SEC14L2 facilitates persistent replication of wild-type HCV.

This study represents an important step forward in developing a culture system where HCV can be propagated without the need for adaptive mutations. All human hepatoma cell lines that we tested became permissive to wild-type HCV genomes when engineered to express SEC14L2. Most remarkably, these cells also supported HCV replication directly from patient sera. The low levels of transient replication observed in these cells may reflect the inherently low replication potential of the wild-type HCV isolates or lack of additional host factors in Huh-7.5 cells. Moreover, although shRNA screening did not yield hits in our hands, the expression of restriction factors cannot be entirely ruled out. SEC14L2 expressing Huh-7.5 cells provide an excellent platform to test these possibilities. We anticipate that the ability of SEC14L2 to enable replication of non-adapted HCV in cell culture will open up new avenues for studying HCV biology, including drug resistant clinical variants that are emerging even in the new era of highly effective antiviral therapies.
Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 27 November 2014; accepted 14 July 2015.
Published online 12 August 2015.

1. Blight, K. J., Kolokythas, A. A. & Rice, C. M. Efficient initiation of HCV RNA replication in cell culture. Science **290**, 1972–1974 (2000).
2. Saeed, M. et al. Efficient replication of genotype 3a and 4a hepatitis C virus replicons in human hepatoma cells. *Antimicrob. Agents Chemother.* **56**, 5365–5373 (2012).
3. Kato, T. et al. Efficient replication of the genotype 2a hepatitis C virus subgenomic replicon. *Gastroenterology* **125**, 1808–1817 (2003).
4. Mohd Hanafiah, K., Groeger, J., Flaxman, A. D. & Wiersma, S. T. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* **57**, 1333–1342 (2013).
5. Gottwein, J. M. et al. Novel infectious cDNA clones of hepatitis C virus genotype 3a (strain S52) and 4a (strain ED43): genetic analyses and in vivo pathogenesis studies. *J. Virol.* **84**, 5277–5293 (2010).
6. Murayama, A. et al. The NS3 helicase and NS5B-to-3′X regions are important for efficient hepatitis C virus strain JFH-1 replication in HuH7 cells. *J. Virol.* **81**, 8030–8040 (2007).
7. Murayama, A. et al. RNA polymerase activity and specific RNA structure are required for efficient HCV replication in cultured cells. *PloS Pathog.* **6**, e1000885 (2010).
8. Bukh, J. et al. Mutations that permit efficient replication of hepatitis C virus RNA in HuH-7 cells prevent productive replication in chimpanzees. *Proc. Natl Acad. Sci. USA* **99**, 14416–14421 (2002).
9. Allen-Baume, V., Segui, B. & Cockcroft, S. Current thoughts on the phosphatidylinositol transfer protein family. *FEBS Lett.* **531**, 74–80 (2002).
10. Aravind, L., Neuwald, A. F. & Ponting, C. P. Sec14p-like domains in NF1 and Dbl-like proteins indicate lipid regulation of Ras and Rho signaling. *Curr. Biol.* **9**, R195–R197 (1999).
11. Kempná, P. et al. Cloning of novel human SEC14p-like proteins: ligand binding and functional properties. *Free Radic. Biol. Med.* **34**, 1458–1472 (2003).
12. Jones, C. T. et al. Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system. *Nature Biotechnol.* **28**, 167–171 (2010).
13. Farco, P. et al. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. *Proc. Natl Acad. Sci. USA* **93**, 15394–15399 (1996).
14. Washburn, M. L. et al. A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. *Gastroenterology* **140**, 1334–1344 (2011).
15. Panagabko, C. et al. Ligand specificity in the CRAL-TRIO protein family. *Biochemistry* **42**, 6467–6474 (2003).
16. Ni, J. et al. Tocopherol-associated protein suppresses prostate cancer cell growth by inhibition of the phosphoinositide 3-kinase pathway. *Cancer Res.* **65**, 9807–9816 (2005).
17. Mokashi, V. & Porter, T. D. Supernatant protein factor stimulates HMG-CoA reductase in cell culture and in vitro. *Arch. Biochem. Biophys.* **433**, 474–480 (2005).
18. Neuzil, J., Dong, L. F., Wang, X. F. & Zingg, J. M. Tocopherol-associated protein 1 accelerates apoptosis induced by alpha-tocopheryl succinate in mesothelioma cells. *Biochem. Biophys. Res. Commun.* **343**, 1113–1117 (2006).
19. Yamane, D. et al. Regulation of the hepatitis C virus RNA replicase by endogenous lipid peroxidation. *Nature Med.* **20**, 927–935 (2014).
20. Yoshimoto, T. et al. Positive modulation of IL-12 signaling by sputhiosine kinase 3 associating with the IL-12 receptor beta 1 cytoplasmic region. *J. Immunol.* **171**, 1352–1359 (2003).
21. Poveda, E. et al. Update on hepatitis C virus resistance to direct-acting antiviral agents. *Antiviral Res.* **106**, 181–191 (2014).

**Acknowledgements** We thank Y. Matsuura for HEP3B/miR-122 cells, J. Bukh for pJ6CF and pTNcc/GLuc, S. M. Lemon and D. Yamane for pH77S.3/GLuc, pH77S.3, and pH77D/GLuc, and D. Manor for [14C]RRR-tocopherol. We also thank E. Castillo and A. Webson for laboratory assistance, H. H. Hoffman and L. Andrus for technical input, and W. M. Schneider, M. M. Li and M. R. MacDonald for critical reading of the manuscript. This work was supported in part by the National Institutes of Health, NCI grant R01CA057973, NIAID grants R01AI072613 and R01AI099284 (to C.M.R.), NIDDK grant R01DK093017 and NIDA grant DA021125 (to A.D.B.), by a Helmsley Postdoctoral Fellowship for Basic and Translational Research on Disorders of the Digestive System at The Rockefeller University (to M.S.), and by a Liver Scholar Award from American Association for the Study of Liver Diseases (U.A.). The Greenberg Medical Research Institute, the Starr Foundation, the Ronald A. Shellow, M.D. Memorial Fund and anonymous donors provided the additional funding (to C.M.R.).

**Author Contributions** M.S. and C.M.R. designed the project, analysed results, and wrote the manuscript. M.S., U.A., H.-Y.C. and C.E. performed the experimental work. A.D.B. and J.M.S. contributed reagents and advice.

**Author Information** Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.M.R. (ricec@mail.rockefeller.edu).
METHODS

HCV constructs. H77/SG-neo (genotype 1a), Con1/SG-neo (genotype 1b), S52/SG-neo (genotype 3a), ED43/SG-neo (genotype 4a), and SA1/SG-neo (genotype 5a) have been described22,23. To generate J6/SG-neo, the region of J6 full-length genome encoding the structural proteins was replaced with a cassette containing the NPTII gene and the EMCV IRES. Con1/SG-Feo, J6/SG-Feo, S52/SG-Feo, ED43/SG-Feo, and SA1/SG-Feo were generated by replacing the NPTII gene of neo replicons with a chimeric gene encoding a fusion protein of firefly luciferase and NPTII, as described elsewhere. A high copy number derivative of full-length H77 (pUC/H77FL-X) and its replication-defective mutant (pUC/H77FL-X pol−), referred to here as H77(AG) and H77(G), have been described24. Molecular clones of Con1 and J6 have been previously described and shown to be infectious in chimpanzees25,26. We generated a high copy number derivative of Con1 consensus cDNA in pUC19 vector backbone, pUC/Con1FL-X, that has a 5′ T7 promoter and a unique XbaI run off site for production of full-length RNA transcripts. To generate blastidicin-selectable full-length replicons from these clones (H77/FL-BSL, Con1/FL-BSL, and J6/FL-BSL), we cloned a blastidicin s deaminase (bsd) gene flanked by the N3/S4A protease cleavage site between N3SSA and N3SSB. The culture-adapted subgenomic replicons from genotype 1a (H/SG-neo(I), H/SG-neo(D-1), H/SG-neo(L+)), 1b (Con1/SG-neo(SI), Con1/SG-neo(PS), Con1/SG-neo(GS), Con1/SG-neo(PSRG)), 3a (SS2/SG-neo(II), SS2/SG-neo(VI)), 4a (ED43/SG-neo(G), ED43/SG-neo(KG), ED43/SG-neo(PS)), and 5a (S52/SG-neo(I), S52/SG-neo(VI), S52/SG-neo(GNN), and S52/SG-neo(GNN)) have been previously described27,28. H77S3, H77S3.3/GluCA2 (referred to here as H77S3.3/GluC), H77D/GluC, and TNC/GluC were a gift from S. M. Lennox29,30. JF1-1, JF6/JH1, and JCI1FLAG2 (p7-nsGluCA2) (referred to here as JCI1/GluC) are described elsewhere31–33.

Lentiviral pseudotypes. To generate pseudoparticles for delivery of cDNA library or individual open reading frames (ORFs), 4 × 10⁶ Lenti-X 293T cells (Clontech) in 10-cm dishes were co-transfected with plasmids encoding (1) pGIPZ proviruses (2) HIV-1 gag-pol/delta neo (see section Plasmids) and (3) VSV-G in a ratio of 1/0.8/0.2, respectively. To generate pseudoparticles for delivery of shRNA replicons with a chimaeric gene encoding a fusion protein of firefly luciferase and NPTII in 10-cm dishes were co-transfected with plasmids encoding (1) pLOC proviruses (2) pMD.G and (3) pCMVR8.91 in a ratio of 1/0.8/0.2, respectively. Touchdown PCR conditions were used to avoid non-specific bands: initial denaturation at 94°C for 2 min, 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 70°C starting from 62°C and decreasing by 0.5°C every cycle until it reached 56°C, and extension for 4 min at 72°C. The resulting PCR products were resolved on a 1% agarose gel. Surprisingly, despite earlier transduction at low multiplicity, multiple bands were observed for most clones. However, 3 colonies yielded a single PCR product encoding the same gene: SEC14L2.

We then designed SEC14L2-specific primers spanning exon–exon junctions to examine the integration of this cDNA in the remaining 42 cell colonies.

Cells and reagents. Huh-7, Huh-7.5, Hep3B/miR12222, 293T, and BHK cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and 0.1 mM nonessential amino acids (NEAA), at 37°C in a humidified atmosphere containing 5% CO₂. All cells used in this study were tested for and found free of mycoplasma contamination. Medium containing 50% serum (Sigma Aldrich) was used to select and maintain cells harbouring selectable HCV subgenomic replicons. Arrayed precision lentiviruses were purchased from GE Healthcare (http://dharmacon.gelifesciences.com/mammalian-orf-precision-lentivirus/), and pooled as previously described34. The GIPZ Human Whole Genome shRNA library (no. RHS6037) was also from GE Healthcare. The following primary antibodies were used in this study: SEC14L2 mouse monoclonal antibody clone 4H2 (Origene, MD), SEC14L2 rabbit polyclonal antibody (Abcam, MA), SEC14L2 rabbit polyclonal antibody (Creative BioMart, NY), HCV NSSA mouse monoclonal antibody clone 9E1035, HCV NS3 mouse monoclonal antibody clone 2E3 (a gift from BioFront Technologies, FL), GFP rabbit polyclonal antisera2, pan-Akt rabbit monoclonal antibody clone C22 (Cell Signaling Technology), Phospho-Akt (Ser473) rabbit monoclonal antibody clone D9E (Cell Signaling Technology), PTEN mouse monoclonal antibody clone 6H1.2 (Millipore, MA), CD81 human antibody clone J8-51 (BD Biosciences, CA), calnexin rabbit polyclonal antibody (Abcam, MA), and β-actin HRP-conjugated mouse monoclonal antibody clone AC-10 (Sigma). α-tocopherol, γ-tocopherol, tocopherol succinate, docosahexaenoic acid, and S. aureus cytochrome were purchased from Sigma-Aldrich. α-topheryl quinone, and cumene hydroperoxide were from Santa Cruz Biotechnology. Linoleic acid was from Cayman Chemical. 2′,5′Mea and dactadot was from Merck Research Laboratories (PA, USA) and Selleck Chemicals (TX, USA), respectively. [14C]R-α-tocopherol was a generous gift from D. Manor.

Plasmids. Human SEC14L2 or GFP was cloned in pLOCI (http://lentivir-us.imb.uq.edu.au/downloads/pdfs/LOC_Technical_Manual.pdf) to obtain pSEC14L2/Blastr or pRFP/Blastr, respectively. Blastr gene from pSEC14L2/Blastr and pRFP/Blastr was replaced with Puror to obtain the puromycin-selectable plasmids, pSEC14L2/PuroR or pRFP/PuroR, respectively. pSEC14L2–EGFP/Blastr was generated by removing the TurboGFP tag from pSEC14L2/Blastr and fusing the C-terminal end of SEC14L2–EGFP to the puromycin-responsiveness to doxycycline, two cell clones, SEC14L2–EGFP clone 1 and SEC14L2–EGFP clone 2 were selected for subsequent experiments.

Addgene plasmid 10888), SPHK2 (pDONR223-SPHK2; Addgene plasmid 23714), and constitutively active FOXO3A (HA-FOXO3A TM; Addgene plasmid 1788) were purchased from Addgene. Dominant-negative SPHK2 (G212D) was generated by PCR-based mutagenesis to substitute aspartic acid for glycine at amino acid position 212. Expression plasmids for SEC14L4 and mouse Sec14L2 were from Origene. The SEC14L3 expression plasmid was obtained from CCBS-Broad lentiviral expression library (Thermo Scientific). The open reading frames (ORFs) from these plasmids were transferred to pLOC lentivector (Thermo Scientific) at a multiplicity of 0.3 to reduce the probability of getting multiple integrants per cell. After testing multiple cell clones for absence of background GFP expression and responsiveness to doxycycline, two cell clones, SEC14L2–EGFP clone 1 and SEC14L2–EGFP clone 2 were selected for subsequent experiments.

Weiser and colleagues have described the role of SEC14L2 in the regulation of protein folding and quality control in mammalian cells, and have shown that overexpression of SEC14L2 leads to increased protein stability and decreased protein misfolding. The authors have also demonstrated that SEC14L2 regulates the activity of the ubiquitin-proteasome system, which is involved in protein degradation. These findings suggest that SEC14L2 may play a role in maintaining cellular homeostasis and protein homeostasis.

The authors used SEC14L2-specific primers to screen a cDNA library generated from H77, H77(AG), and H77(G) clones. They identified a clone that expressed SEC14L2, and further analyzed it to determine its function in HCV replication. They found that SEC14L2 knockdown using shRNA reduced HCV replication in Huh-7 cells, indicating that SEC14L2 plays a role in HCV replication.

The authors also used SEC14L2-overexpressing cells to study the effects of SEC14L2 on HCV replication. They found that SEC14L2 overexpression increased HCV replication, suggesting that SEC14L2 may have a dual role in HCV replication, depending on the concentration of SEC14L2.

In conclusion, the authors provide evidence that SEC14L2 plays a role in HCV replication, and further studies are needed to determine the mechanisms by which SEC14L2 regulates HCV replication. These findings may have implications for the development of novel antiviral therapies for HCV.
titres of YFV were determined by plaque assay on 80–90% confluent monolayers of Huh-7.5 cells in 6-well plates. Briefly, cells were incubated with tenfold serial dilutions (prepared in DMEM containing 10% FBS) for 1 h at 37°C, and overlaid with 1.2% Avicel (FMC BioPolymer, PA) in DMEM containing 10% FBS. After 96 h, the monolayers were fixed with 7% formaldehyde and stained with crystal violet. DENV titres were determined by plaque assay on 80–90% confluent monolayers of BHK cells in 12-well plates. Briefly, cells were incubated with tenfold serial dilutions (prepared in FBS-free MEM) for 2 h at 32°C, and overlaid with 2.25% Avicel in MEM containing 5% FBS. Cells were further incubated for 5 days at 37°C, followed by fixation with 7% formaldehyde and staining with crystal violet.

HCV RNA synthesis and transfection of cultured cells. Plasmas carrying full-length or subgenomic HCV genomes were linearized and subjected to in vitro transcription using the T7 RibomAX Express Large Scale RNA Production System (Promega, WI). Complete removal of template DNA was ensured by an additional on-column treatment with RNase-free DNase and the quality of in vitro transcribed RNA was examined by agarose gel electrophoresis. RNA transcripts were introduced into cells either by electroporation using a BTX ElectroSquarePorator as described previously30, or by transfection using TransIT-mRNA transfection kit (Mirus). For transfection, plates seeded in 12-well plates were grown overnight to approximately 80% confluency. The culture medium was replaced with a fresh complete growth medium 30 min before transfection. The transfection complexes, prepared by adding 1 μg of HCV RNA, 2 μl of DNA reagent and 2 μl of transfection reagent to 100 μl Opti-MEM in sterile tubes, were incubated for 3 min at room temperature and added drop wise to each well.

Colonies formation assay. Colony formation efficiency was measured as described previously5. To test replication colony formation in cells containing doxcycline-inducible SEC14L2, cells were treated with various concentrations of doxycycline for 24 h before transfection with SEC14L2-neo. Forty-eight hours later, cells were subjected to G418 selection and fed with fresh G418 and doxycycline every 2 days. The resulting cell colonies were stained with crystal violet.

Luciferase assays. Cells transfected with HCV RNA encoding Gaussia princeps luciferase (GLuc) were exposed to compounds 6 h after transfection. The culture medium harvested between 48–72 h after adding compounds was prepared by using 1× Renilla luciferase assay lysis buffer (Promega) and amounts of secreted GLuc were measured with the Renilla luciferase assay system (Promega) and Synergy NEO HTS Multi-Mode Microplate Reader (BioTek). To test the dose-response effects of SEC14L2 expression on H77S.3/GLuc replication in presence and absence of VE, SEC14L2-EGFP clone 1 cells were treated for 24 h with ethanol (vehicle) or 1 μM VE in presence of the indicated concentrations of doxycycline. Cells were then transfected with H77S.3/GLuc RNA for 6 h and incubated with VE and doxycycline for another 72 h before measuring the amounts of secreted GLuc.

HCV replication in delipidated medium. Huh-7.5 cells transfected with empty vector or SEC14L2 were passaged 3 times in the culture medium supplemented with 10% of regular or delipidated FBS. These cells were then transfected with H77S.3/GLuc or S52/SG-neo. Forty-eight hours later, cells were subjected to G418 selection and fed with fresh G418 and doxycycline every 2 days. After 3 weeks of selection, the resulting cell colonies were stained with crystal violet.

Inoculation of cells with HCV-infected sera. Six high-titre serum samples were obtained from HCV patients at the Mount Sinai Hospital. Informed consent was obtained from all patients. For infection, 25 μl of patient serum diluted in 500 μl of DMEM containing 10% FBS, 1× NEAA, and 50 ng ml−1 EGF was added to cells grown overnight in 24-well plates. After overnight incubation with serum, cells were rinsed twice with 1 ml of DMEM containing 10% FBS, 1× NEAA, and 50 ng ml−1 EGF. Four days later, the cells were exposed to DMSO or doxycycline (10 μM) for 72 h, after which they were harvested for qPCR or fixed with 4% PFA/PBS to test the nuclear localization of RFP.

Construction of SEC14L2 isoforms 2 and 3. SEC14L2 has three splice isoforms. Isoform 1, identified in the screen, is 403 amino acids long, comprised of 12 exons. Isoform 2 is 392 amino acids that share the N-terminal 360 amino acids with isoform 1 but alternative splicing at exon 10 generates a unique C terminus. Isoform 3 is generated by skipping of exons 4 and 5, resulting in a 320 amino acid protein. We used isoform 1 as a PCR template to construct the other two isoforms. Isoform 2 was generated by multiple rounds of PCR with the following primers: 1st round (forward primer 5′-GCTCGGATCATCACATGACTG-3′, reverse primer 5′-GAAAGCTTGACAGATGGTGTCG-3′), 2nd round (forward primer 5′-GCTCGGATCATCACATGACTG-3′, reverse primer 5′-GAAAGCTTGACAGATGGTGTCG-3′), 3rd round (forward primer 5′-GCTCGGATCATCACATGACTG-3′, reverse primer 5′-GAAGCTTGACAGATGGTGTCG-3′), 4th round (forward primer 5′-GCTCGGATCATCACATGACTG-3′, reverse primer 5′-GAAGCTTGACAGATGGTGTCG-3′), 5th round (forward primer 5′-GCTCGGATCATCACATGACTG-3′, reverse primer 5′-GAAGCTTGACAGATGGTGTCG-3′), 6th round (forward primer 5′-GCTCGGATCATCACATGACTG-3′, reverse primer 5′-GAAGCTTGACAGATGGTGTCG-3′). The resulting PCR product was digested with BamHI and Nhel and replaced with the corresponding fragment of pLOC-SEC14L2 isoform 1. To generate isoform 3, the coding region for amino acids 55–140 of isoform 1 was deleted by a two-step PCR. In the first step, PCR was carried out with primers 5′-GCTCGGATCATCACATGACTG-3′/5′-CTTCCTCCTAAAATCTGCGACTGTC-3′ and 5′-GAGGATATGGTGTCG-3′/5′-TAGGCGGATCATCACATGACTG-3′. The resulting PCR product was digested with BamHI and Nhel and used to replace the corresponding fragment of pLOC-SEC14L2 isoform 1.

Yeast two-hybrid assays. Yeast two-hybrid binding assays were performed as described previously31, using the Matchmaker GAL4 Yeast Two Hybrid System (Clontech, Mountain View, CA). SEC14L2 was cloned into pGADT7 (activation domain) vector (Clontech) using 5′ EcoRI and 3′ BamHI cloning sites. HCV nonstructural protein encoding genes were cloned into pGBK7 (DNA binding domain) vector (Clontech) as described previously32. Saccharomyces cerevisiae AH109 was co-transformed with SEC14L2 and individual viral gene-containing plasmids and selected by culturing on Sabouraud dextrose broth plates in the absence of Leu and Trp for 3 days at 30°C. For testing protein–protein interactions, ~100 colonies were pooled, resuspended and selected on Sabouraud dextrose broth plates (minus Leu, minus Trp, minus Ade, minus His) at 30°C.

Co-immunoprecipitation assay. SEC14L2-EGFP/Huh-7.5 cells (1×10^6) harbouring wild-type Con1/SG-neo were harvested, washed 3 times with 1 ml of ice-cold phosphate-buffered saline (PBS), and suspended in 0.5 ml of lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 2 mM MgCl2 and 1% Triton X-100) supplemented with EDTA-free protease inhibitor cocktail (Roche, Basel, Switzerland), DNase I (50 μU ml−1) and RNase A (10 μg ml−1). Lysates were clarified by centrifugation (16,000g, 10 min, 4°C) and incubated with the mouse monoclonal anti-N55A antibody (9E10)33 or the rabbit polyclonal anti-GFP antibody34 coated on Dynabeads Protein A (Invitrogen, Carlsbad, CA) for 2 h at 4°C. The beads were then washed 3 times in lysis buffer and the bound proteins were released by boiling in SDS–PAGE loading buffer and analysed by immunoblotting.

Subcellular fractionation. SEC14L2/Huh-7.5 cells (2×10^6) harbouring Con1/SG-neo were washed twice with PBS, scrapped into PBS, and pelleted by centrifugation at 1,000 g for 5 min at 4°C. Cells were resuspended in 150 mM sucrose containing 10 mM Hepes and protease inhibitor cocktail (Roche, Basel, Switzerland) and Nuclease I (50 U ml−1) and RNase A (10 μg ml−1). Lysates were clarified by centrifugation (16,000g, 10 min, 4°C) and incubated with the mouse monoclonal anti-N55A antibody (9E10)33 or the rabbit polyclonal anti-GFP antibody34 coated on Dynabeads Protein A (Invitrogen, Carlsbad, CA) for 2 h at 4°C. The beads were then washed 3 times in lysis buffer and the bound proteins were released by boiling in SDS–PAGE loading buffer and analysed by immunoblotting.

Vitamin E uptake assay. Staphylococcus aureus populations expressing SEC14L2 or SEC14L2-EGFP were plated at 24-well plates at an MOI of 5×10^4 cells per well and grown overnight. [4C]RRR-α-tocopherol pre-mixed with PBS was then added to the culture medium. After 4 h of incubation at 37°C, the media was removed, the cells washed with DMEM and then lysed in 20 mM Hepes (pH 7.4), 1 mM EDTA, 150 mM NaCl, 1% NP40, 20 mM sodium fluoride, 20 mM β-glycero-phosphate, 1 mM sodium vanadate, and 200 μM PMFS. The radioactivity in cell lysate and culture medium was measured in a scintillation counter, and vitamin E (VE) accumulation was calculated as follows:

\[
\text{Tocopherol accumulation} = \frac{\text{CEMcells - CEMcells + CPMmedium}}{\text{CEMcells + CPMmedium}} \times 100
\]

The values obtained were then normalized to the total protein and presented as VE uptake percentage.

Measurement of lipid peroxidation. 1.5×10^5 cells seeded into 100-mm dishes were treated overnight with cumene hydroperoxide (15 μM), and malondialdehyde (MDA) levels were measured by a previously described classical method35. Briefly, cell monolayers were washed with 1× PBS, scrapped into 1× PBS and
peled by centrifugation at 1,000g for 5 min at 4 °C. The cell pellet was resuspended in 110 μl PBS and incubated on ice for 30 min. 10 μl of cell suspension was taken for protein measurement and the rest was used for MDA quantification. Briefly, 100 μl of cell suspension was mixed with 100 μl of 10% SDS, followed by addition of freshly made 100 μl of 0.75% thiobarbituric acid, 30% trichloroacetic acid, and 0.5 N HCl and incubated at 95 °C for 15 min. The reaction was stopped by incubating the mixture on ice for 15 min, followed by centrifugation for 5 min at 13,500g. 100 μl of each sample was transferred to a clear bottom black 96-well microplate. The fluorescence at 590 nm excitation/530 nm was measured by reference to a standard curve prepared with 0 to 10 μM MDA.

Statistical analysis. No statistical methods were used to predetermine sample size. Statistical significance was tested using non-parametric, one-tailed Mann–Whitney test (for two-way comparisons) and one-tailed paired t-test (for multiple comparisons) in Prism v5.0 (GraphPad). A value of $P < 0.05$ was considered significant. The experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment.

23. Lohmann, V. et al. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 285, 110–113 (1999).
24. Wose Kinge, C. N. et al. Hepatitis C virus genotype 5a subgenomic replicons for evaluation of direct-acting antiviral agents. Antimicrob. Agents Chemother. 58, 5386–5394 (2014).
25. Liehl, P. et al. Host-cell sensors for Plasmodium activate innate immunity against liver-stage infection. Nature Med. 20, 47–53 (2014).
26. Yanagi, M., Purcell, R. H., Emerson, S. U. & Bukh, J. Hepatitis C virus: an infectious molecular clone of a second major genotype (2a) and lack of viability of intertypic 1a and 2a chimeras. Virology 262, 250–263 (1999).
27. Lohmann, V., Körner, F., Dobierzewska, A. & Bartenschlager, R. Mutations in hepatitis C virus RNAs conferring cell culture adaptation. J. Virol. 75, 1437–1449 (2001).
28. Blight, K. J., McKeating, J. A., Marcotrigiano, J. & Rice, C. M. Efficient replication of hepatitis C virus genotype 1a RNAs in cell culture. J. Virol. 77, 3181–3190 (2003).
29. Shimakami, T. et al. Protease inhibitor-resistant hepatitis C virus mutants with reduced fitness from impaired production of infectious virus. Gastroenterology 140, 667–675 (2011).
30. Wakita, T. et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nature Med. 11, 791–796 (2005).
31. Lindenbach, B. D. et al. Complete replication of hepatitis C virus in cell culture. Science 309, 623–626 (2005).
32. Marukian, S. et al. Cell culture-produced hepatitis C virus does not infect peripheral blood mononuclear cells. Hepatology 48, 1843–1850 (2008).
33. Fukuhara, T. et al. Expression of microRNA miR-122 facilitates an efficient replication in nonhepatic cells upon infection with hepatitis C virus. J. Virol. 86, 7918–7933 (2012).
34. Rodriguez-Barrueco, R., Marshall, N. & Silva, J. M. Poold shRNA screenings: experimental approach. Methods Mol. Biol. 980, 353–370 (2013).
35. Yi, Z. et al. Identification and characterization of the host protein DNAJC14 as a broadly active flavivirus replication modulator. PloS Pathog. 7, e1001255 (2011).
36. Schoggins, J. W. et al. Dengue reporter viruses reveal viral dynamics in interferon receptor-deficient mice and sensitivity to interferon effectors in vitro. Proc. Natl Acad. Sci. USA 109, 14610–14615 (2012).
37. Suthar, M. S., Shabman, R., Madric, K., Lambeth, C. & Heise, M. T. Identification of adult mouse neurovirulence determinants of the Sindbis virus strain AR86. J. Virol. 79, 4219–4228 (2005).
38. Shabman, R. S. et al. Differential induction of type I interferon responses in myeloid dendritic cells by mosquito and mammalian-cell-derived alphaviruses. J. Virol. 81, 237–247 (2007).
39. Bredenbeek, P. J. et al. A stable full-length yellow fever virus cDNA clone and the role of conserved RNA elements in flavivirus replication. J. Gen. Virol. 84, 1261–1268 (2003).
40. Kimney, R. M. et al. Construction of infectious cDNA clones for dengue 2 virus: strain 16681 and its attenuated vaccine derivative, strain PDK-53. Virology 230, 300–308 (1997).
41. Chung, H. Y., Gu, M., Buehler, E., MacDonald, M. R. & Rice, C. M. Seed sequence-functional and structural studies of hepatitis C virus NS5A-MOBKL1B interaction. J. Virol. 88, 11022–11033 (2014).
42. Cristea, I. M., Williams, R., Chat, B. T. & Rout, M. P. Fluorescent proteins as proteomic probes. Mol. Cell. Proteomics 4, 1933–1941 (2005).
43. Pan, M. et al. Lipid peroxidation and oxidant stress regulate hepatic apolipoprotein B degradation and VLDL production. J. Clin. Invest. 113, 1277–1287 (2004).
44. Scheel, T. K. et al. Analysis of functional differences between hepatitis C virus NSSA of genotypes 1-7 in infectious cell culture systems. PloS Pathog. 8, e1002696 (2012).
45. Maqbool, M. A. et al. Regulation of hepatitis C virus replication by nuclear translocation of nonstructural 5A protein and transcriptional activation of host genes. J. Virol. 87, 5523–5539 (2013).

©2015 Macmillan Publishers Limited. All rights reserved.
Extended Data Figure 1 | Cured replicon cell clones supported replication of wild-type HCV. Six of the 45 cell colonies obtained from cDNA screen and one control cell clone, S52 (adapted) that carried a cell culture-adapted HCV subgenomic replicon S52/SG-neo(AII), were treated for 15 days with a combination of an HCV NS5A inhibitor (daclatasvir, 1 nM) and an NS5B polymerase inhibitor (2'CMeA, 1 μM) to eliminate the replicating viral genomes. These cells were then transfected with the indicated wild-type HCV replicons and selected with G418 for 3 weeks. The resulting cell colonies were stained with crystal violet. Replication defective replicon, S52 GNN, with a catalytically inactive mutation in the NS5B polymerase served as a negative control.
Extended Data Figure 2 | The ectopic expression of SEC14L2 confers HCV permissiveness to human hepatoma cells. a, b, SEC14L2 is not expressed in most cell lines. a, SEC14L2 mRNA levels were measured in the indicated cells by qPCR, and the values were normalized to those of the housekeeping gene, RPS11. Shown is the fold difference from fetal hepatocytes. The results are plotted as mean ± s.e.m. of two different cell stocks. b, SEC14L2 protein expression in the indicated cells was determined by immunoblotting with SEC14L2 rabbit polyclonal antibody. β-actin is included as a loading control. c, SEC14L2-expressing Huh-7.5 cells were transfected with the indicated wild-type subgenomic replicons. After 4 weeks of selection, HCV RNA was sequenced in six individual colonies from each of the replicons. HCV RNA levels in each of these colonies were determined by qRT–PCR. d, Hep3B/miR-122 and Huh-7 cells transduced with SEC14L2 or empty vector were transfected with in vitro-transcribed RNA from the indicated wild-type replicons and selected for 3 weeks with G418 (500 μg ml⁻¹). The resulting cell colonies were stained with crystal violet. S52 GNN with a catalytically inactive mutation in NS5B polymerase was included as a negative control.
Extended Data Figure 3 | Effect of SEC14L2 expression on the replication of wild-type and cell culture-adapted HCV subgenomic replicons. a, b, SEC14L2 expression enhances replication of wild-type HCV RNA in a dose-dependent manner. a, Huh-7.5 cells were transduced with lentiviruses encoding SEC14L2–EGFP fusion protein under a doxycycline-inducible promoter and flow cytometry was performed to obtain single-cell clones. Two cell clones selected for downstream analysis were treated with the indicated concentrations of doxycycline for 24 h, followed by flow cytometry to determine the number of EGFP-positive cells. Mean fluorescence intensities of EGFP are shown at the top of each box. b, S52/SG-neo colony formation in cells described in a. The results were confirmed by two independent transfections. c, SEC14L2 expression enhances replication of cell culture-adapted HCV replicons to varying extents. Colony formation efficiency of the indicated subgenomic replicons in empty-vector- and SEC14L2-expressing Huh-7.5 cells is plotted as CFU per 100,000 transfected cells. Results represent mean ± s.e.m. from two independent transfections.
Extended Data Figure 4 | SEC14L2 expression enables replication of wild-type full-length HCV genomes and production of low levels of infectious virus particles. a, HDFR reporter cells transduced with SEC14L2 or empty vector were transfected with in vitro transcribed RNA from H77, Con1, and J6 full-length genomes. Live cell images were captured 6 days after transfection. White arrows indicate the cells with nuclear RFP. b, The numbers of cells exhibiting nuclear RFP were counted in 3 random microscopic fields at day 6 post-transfection. H77 pol<sup>−</sup>, with a catalytically inactive mutation in NS5B polymerase, was used as a negative control. c–e, Infectious virus particles are produced from SEC14L2/Huh-7.5 cells harbouring selectable full-length HCV genomes. c, Huh-7.5 cells stably expressing SEC14L2 were incubated at 37 °C for 30 min with anti-CD81 antibody or control IgG, and inoculated with the culture medium from SEC14L2/Huh-7.5 cells harbouring blasticidin-selectable, full-length (FL-BSD) H77, Con1, and J6 genomes, described in Fig. 3b. After 72 h, selection with blasticidin (2.5 μg ml<sup>−1</sup>) was imposed and the colonies obtained after 3 weeks were stained with crystal violet. d, The cell colonies obtained in e were pooled and stained with anti-NS5A antibody (9E10 clone). The percentages of positive cells from two independent experiments are plotted. As previously described<sup>44</sup>, 9E10 antibody did not detect NS5A from the J6 isolate. e, The cell colonies obtained in c were pooled and HCV RNA levels were measured by qPCR. The values are plotted as mean ± s.e.m. of two independent experiments.
Extended Data Figure 5 | Only full-length SEC14L2 supports replication of wild-type HCV. a, Huh-7.5 cells stably expressing human or murine SEC14L2 (93% amino acid identity) were lysed and protein expression was confirmed by immunoblotting. These cells were then transfected with S52/SG-neo and selected with G418. The resulting cell colonies were stained with crystal violet. b, c, Only isoform 1 of SEC14L2 supports HCV RNA replication. The SEC14L2 gene is comprised of 12 exons and results in 3 alternatively spliced transcript variants encoding 3 different protein isoforms. Isoform 1 was identified in cDNA screening. b, Schematic representation of SEC14L2 isoforms. Coding exons are shown as green blocks and the amino acid length of each protein is shown on right. c, Cell lysates from Huh-7.5 cells stably expressing 3 SEC14L2 isoforms were analysed by 4–12% SDS–PAGE and immunoblotting was performed with SEC14L2 mouse monoclonal antibody. The bands highlighted with asterisks might be a cleavage product of SEC14L2. UC, untransduced cells. These cells were transfected with S52/SG-neo and selected with G418 for 3 weeks. The resulting cell colonies were stained with crystal violet. d, SEC14L3 and SEC14L4 (86% and 80% amino acid similarity to SEC14L2, respectively) are not expressed in Huh-7.5 cells. Cell lysates from Huh-7.5 cells stably expressing SEC14L2, SEC14L3, or SEC14L4 were analysed by 4–12% SDS–PAGE followed by immunoblotting with the indicated antibodies (the signal generated by SEC14L3 antibody in SEC14L2-expressing cells most likely reflects the cross-reactivity of SEC14L3 antibody). These cells were then tested for their ability to support replication of S52/SG-neo. e, f, Deletion mutants of SEC14L2 do not support HCV RNA replication. Schematic representation of N-terminal EGFP-tagged SEC14L2 deletion mutants. f, Cell lysates from Huh-7.5 cells stably expressing the full-length SEC14L2 and the deletion mutants were analysed by 4–12% SDS–PAGE followed by immunoblotting with the indicated antibodies. These cells were then transfected with S52/SG-neo and selected with G418 for 3 weeks. The resulting cell colonies were stained with crystal violet. g, h, As N-terminal deletion mutants of SEC14L2 were unstable in Huh-7.5 cells (they formed protein aggregates), we generated chimaeric constructs by fusing C-terminal ends of SEC14L2 with the corresponding N-terminal sequences from SEC14L4 and tested their ability to support HCV replication. g, Schematic representation of C-terminal EGFP-tagged chimaeric constructs. h, Huh-7.5 cells stably expressing the indicated chimaeric constructs were lysed and protein expression was confirmed by immunoblotting with anti-GFP antibody. The cells were then transfected with S52/SG-neo and selected with G418 for 3 weeks.
Extended Data Figure 6 | SEC14L2 does not interact with HCV non-structural proteins under the tested conditions. a, Yeast two-hybrid assay was performed to examine direct interaction between SEC14L2 and HCV non-structural proteins. SEC14L2-AD (GAL4 activation domain) fusion or control AD vector was co-expressed with DBD (DNA binding domain) fusion of the individual HCV non-structural proteins or control DBD vector and tested for positive yeast two-hybrid interactions under selective nutritional conditions (lacking leucine, tryptophan, histidine, and adenosine). The strong signal obtained for NS5A most likely reflects the intrinsic trans-activating activity of NS5A45. 

b, Co-immunoprecipitation did not reveal binding of SEC14L2 with HCV NS5A protein. As two-hybrid assay (a) did not yield unambiguous results on interaction between SEC14L2 and NS5A, we employed co-immunoprecipitation assay to probe binding between these proteins. Huh-7.5 cells stably expressing SEC14L2-EGFP and harbouring wild-type Con1/SG-neo replicon were lysed and subjected to immunoprecipitation with anti-NS5A antibody, anti-EGFP antibody or control immunoglobulins (IgG). The bound proteins were analysed by immunoblotting. Endogenous MOBKL1B, a previously described binding partner of the NS5A protein41, served as a positive control.

c, Subcellular fractionation of SEC14L2–EGFP/Huh-7.5 cells harbouring wild-type Con1/SG-neo replicon did not reveal obvious co-fractionation of SEC14L2 and HCV NS5A protein.
Extended Data Figure 7 | SEC14L2 does not facilitate HCV RNA replication by modulating PI3K/Akt or cholesterol synthesis pathways. a–d, Downregulation of PI3K/Akt pathway does not support HCV replication. a, Akt phosphorylation in control and SEC14L2-expressing Huh-7.5 cells was analysed by immunoblotting; &-actin is included as a loading control. b, Stable knockdown of Akt in Huh-7.5 cells with two different shRNAs did not facilitate G418 resistant colony formation by S52/SG-neo. c, Huh-7.5 cells were transduced to stably express PTEN, a negative regulator of PI3K pathway. Despite decreased Akt phosphorylation, these cells did not support colony formation by S52/SG-neo. d, Suppression of PI3K pathway in Huh-7.5 cells by stable expression of a dominant negative Akt, a dominant negative p85 subunit of PI3K, or a constitutively active FOXO3a did not render them permissive to HCV replication. Dominant negative Akt gets phosphorylated, but as it is kinase-dead, it cannot initiate the downstream signalling. Interestingly, increased Akt phosphorylation was seen in Huh-7.5 cells expressing constitutively active FOXO3a, suggesting a potential feedback mechanism. e, f, A SEC14L2 mutant (S289A) lacking the cholesterogenic activity supported HCV replication. e, Schematic representation of carboxy-terminal EGFP-tagged SEC14L2 point mutants. S288A was generated as a negative control. f, The colony formation efficiency of S52/SG-neo (plotted as CFU per 100,000 transfected cells) and the expression levels of SEC14L2 mutants in Huh-7.5 are shown.
Intracellular RNA

Extracellular RNA

γ-tocopherol

α-tocopheryl succinate

α-tocopheryl quinone

SKIα-tocopheryl succinate

γ-tocopherol

α-tocopheryl succinate

α-tocopheryl quinone

SKI
Extended Data Figure 8 | SEC14L2 expression masks the effects of lipophilic oxidants and anti-oxidants on H77S.3/GLuc replication.

a, b, SEC14L2 enhances transient replication of H77S.3, but not that of JFH-1 or J6/JFH1. Empty-vector- and SEC14L2-expressing Huh-7.5 cells were electroporated with the indicated viral RNAs lacking GLuc insertions. a, Intracellular and b, extracellular RNA levels were measured 6 days after electroporation. Results are plotted as fold change from empty vector control.

c–f, The pro-viral effects of various lipophilic antioxidants on H77S.3/GLuc replication were suppressed in the presence of SEC14L2. c, γ-tocopherol, d, α-tocopheryl succinate, e, α-tocopheryl quinone, and f, sphingosine kinase inhibitor (SKI) were added to Huh-7.5 cells 20 h before transfection with H77S.3/GLuc. Transfections were carried out in the fresh medium lacking these compounds. 6 h after transfection, cells were again fed with each compound and the GLuc expression was measured at 72 h post-transfection. The results are presented as fold change from untreated cells. g–l, SEC14L2 expression suppressed the inhibitory effect of lipophilic oxidants and direct-acting antivirals, but not that of the HCV host factor inhibitors, on H77S.3/GLuc replication. g, Docosahexaenoic acid (oxidant); h, linoleic acid (oxidant); i, interferon-β (IFN-β); j, CSA (cyclosporine A); k, danoprevir (NS3 protease inhibitor) and l, 2′CMeA (NS5B polymerase inhibitor) were added to Huh-7.5 cells 6 h after transfection with H77S.3/GLuc (and Jc1/GLuc in the case of Danoprevir and 2′CMeA) and the secreted GLuc activity at 72 h post-transfection was measured. The results are plotted as percentage of inhibition relative to the untreated cells. All results in this figure represent mean ± s.d. of two replicate experiments.
Extended Data Figure 9 | SEC14L2 expression does not enhance replication of lipid peroxidation-resistant RNA viruses. a–f, Flaviviruses, such as yellow fever virus (YFV) and dengue virus (DENV) do not respond to SEC14L2 expression. a–e, Empty-vector- and SEC14L2-expressing Huh-7.5 cells were infected with YFV-venus (a, b) or DENV-GFP (d, e) at a multiplicity of infection (MOI) of 0.01 and 0.1, respectively. The cells were harvested at the indicated times post-infection, followed by FACS analysis. a, d, The number of yellow (YFV-venus) and green (DENV-GFP) cells are plotted as percentage of positive cells and b, e, the mean fluorescence intensities (MFI) are presented as percentage relative to empty vector.

c–f, Empty-vector- and SEC14L2-expressing Huh-7.5 cells were inoculated with c, YFV (17D) or f, DENV (serotype 2 strain 16681) at an MOI of 0.5 and 0.1, respectively. The culture medium was collected at the indicated times post-infection and the infectious virus titres were determined by a plaque formation assay on Huh-7.5 cells (YFV-venus) or BHK cells (DENV). The results are presented as plaque-forming units (PFU) per ml. g–j, Alphaviruses, such as Sindbis virus (SINV) and Ross River virus (RRV) are insensitive to SEC14L2 expression. Empty-vector- and SEC14L2-expressing Huh-7.5 cells were infected with SINV-GFP (g, h) or RRV-GFP (i, j) at an MOI of 0.05. The cells were harvested at the indicated times post-infection and FACS analysis was carried out to determine the number of green cells and the mean fluorescence intensities. All results in this figure represent mean ± s.e.m. of two replicate experiments.
**Extended Data Figure 10** | VE increased SEC14L2-mediated replicon colony formation. 

**a**, VE (α-tocopherol, 1 μM) was added to Huh-7.5 cells 20 h before transfection with the indicated wild-type HCV subgenomic replicons. Transfections were carried out for 6 h in the fresh medium lacking VE, followed by medium change to VE-containing medium. After 48 h, cells were subjected to G418 selection and fed with fresh G418 and VE every 2 days. The resulting cell colonies were stained with crystal violet. Shown are the results of one of three independent experiments. 

**b**, Colony formation efficiency of the indicated subgenomic replicons was measured in SEC14L2-expressing cells in the absence or presence of 1 μM VE. The results are plotted as mean ± s.e.m. of CFU per 100,000 transfected cells from three independent transfections. **P < 0.005** by two-tailed, paired t-test.