SUPPLEMENTARY DATA

EXPERIMENTAL PROCEDURES

Reagents and antibodies. Human recombinant apoE3, apoE2, apoE4, chlorpromazin (CPZ), filipin (FPN), Heparinase III and the liver X receptor (LXR) agonist T0901317 were obtained from Sigma-Aldrich®. Human purified VLDL and IDL/LDL were purchased from BioVision. Mouse monoclonal anti-apoE (ab1906), mouse monoclonal anti-βactin (ab8226), rabbit monoclonal anti-LDLR (ab52818), rabbit monoclonal anti-LRP1 (ab92544), and rabbit monoclonal anti-SR-B1 (ab52629), antibodies were obtained from Abcam. The mouse monoclonal anti-core (MA1-080) antibody was obtained from ThermoFisher Scientific. Mouse monoclonal anti-ABCA1 (sc-58919) and rabbit polyclonal anti-apoA1 (sc-30089) antibodies were obtained from Santa Cruz Biotechnology. Rabbit polyclonal anti-ABCG1 (13578-1-AP) was obtained from Proteintech™. The sheep polyclonal anti-NS5A antibody was kindly provided by Pr Mark Harris (University of Leeds, UK).

RNA interference assays. Specific pools of siRNAs targeting LDLR (L-011073-00), LRP1 (L-004721-00), SR-B1 (L-010592-00), ABCA1 (L-004128-00) and non-targeting control siRNA (D-001810-10-05) were purchased from Dharmacon Inc. Specific siRNA targeting ABCG1 (s18482) was purchased from Ambion, Life Technologies™ . SiRNAs were reverse-transfected using Lipofectamine RNAiMax (Life Technologies™). Three days post-transfection, target gene expression was assessed by Western blot analysis.

Cell proliferation assay. HCV replicating cells were treated with increasing amount of LF-apoE3 (2 to 10 μg/mL) for 24h or 48h, as described above. In parallel, a positive control was performed using DMSO (2 to 10%) to slow down cell proliferation. Cell proliferation was assessed by analyzing the ability to metabolize 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich®), as described (1).

Iodixanol density gradient ultracentrifugation. HCV replicating cells (grown in 10% FBS medium or grown in serum-free medium) or PHH (grown in serum-free medium) were treated with LF-apoE3 at 6 μg/mL or PBS (CTRL). Culture media were collected 48h post-treatment. Lipoprotein separation was performed using a flotation iodixanol gradient protocol adapted from Yee *et al*. (30). A 20% iodixanol/sample solution was prepared by diluting 2.25 ml Optiprep™ (Sigma-Aldrich®) with 4.5 ml of cell supernatant. Iodixanol solutions 9% and 12% were prepared by diluting 9 ml of 60% iodixanol (Optiprep™) with 51 ml of 0.1 M HEPES-buffered saline (0.85 g NaCl in 90 ml distilled water, with 10 ml of 1 M HEPES added, adjusted to pH 7.4) and 12 ml Optiprep™ with 48 ml HEPES-buffered saline, respectively. The gradient was prepared by placing 3 mL of the 9% solution in a polycarbonate centrifugation tube (number 355603, 10.4 ml). This was under-layered with 3 ml of the 12% solution and with 3 ml of the 20% iodixanol/sample solution. The tube was then carefully filled to the top with HEPES-buffered saline and capped. The tubes were centrifuged using Ti-70.1 rotor at 65,000 rpm (388,000 g) at 4°C for 6 h in an Beckman Optima™ LE-80k ultracentrifuge (Beckman Coulter) (slow acceleration and deceleration). Fractions were collected using the Biologic LP™ system (Biorad) that pumped 0.5 mL per fraction *via* a syringe river set at 60 mL/h. ApoAI and apoE were detected in the 21 fractions harvested by WB analysis.

FIGURE LEGENDS

Supplementary figure S1. Schematic representation of viral genomes used in this study

JFH1 corresponds to the full-length HCV Japanese fulminant hepatitis 1 genome (genotype 2a). Luc-JFH1 is the corresponding virus carrying the firefly luciferase reporter gene under the control of HCV IRES and JFH1 structural and non-structural proteins genes under the control of the IRES of encephalomyocarditis virus (EMCV IRES). Luc-JFH1ΔE1E2 is a Luc-JFH1-derived virus lacking the coding region for envelope glycoproteins E1 and E2. Jc1 is a chimeric HCV genome, which consists of J6CF structural protein and JFH1 non-structural protein segments. Luc-Jc1 represents the correspondent virus carrying the firefly luciferase reporter gene. The bicistronic, subgenomic replicon genotype 1b is composed of the HCV 5′ non-translated region (NTR) plus nt 342–377 of the core-encoding region, the firefly luciferase gene sequence (Luc), the ubiquitin-encoding sequence (Ubi),

the *neo* gene (neomycin resistance), the EMCV IRES, the coding region of the HCV non-structural proteins NS3–NS5B containing cell culture-adaptive mutations (black arrow) and the HCV 3′ NTR.

Supplementary figure S2. LF-apoE3 treatment does not affect Huh7.5.1 cell proliferation. HCV replicating cells were treated with LF-apoE3 (2 to 10 µg/mL) or DMSO (2 to 10%) as a control for cell proliferation inhibition, for 24h or 48h. Cell proliferation was assessed by MTT assay. Means ± SD from three independent experiments performed in triplicate are shown. .

Supplementary figure S3. LF-apoE3 impairs HCV replication independently of serum components. (A) FBS does not interfere with LF-apoE3 effect on HCV replication. LF-apoE3 was diluted in DMEM medium containing 10, 2 or 0% of FBS and added to HCV replicating cells for 24h. Viral replication was determined by measuring luciferase activity (B) Lipoprotein-bound apoE has no effect on HCV replication. HCV replicating cells were treated with VLDL or IDL/LDL for 48h. Viral replication was assessed by measuring luciferase activity. Results are presented as % luciferase activity relative to CTRL non-treated HCV replicating cells (100%). Means ± SD from five (A) or three (B) independent experiments performed in triplicate are shown. . *$p < 0.01$; ** $p < 0.001$

Supplementary figure S4. Blocking apoE recycling does not impact LF-apoE3's effects on HCV replication.

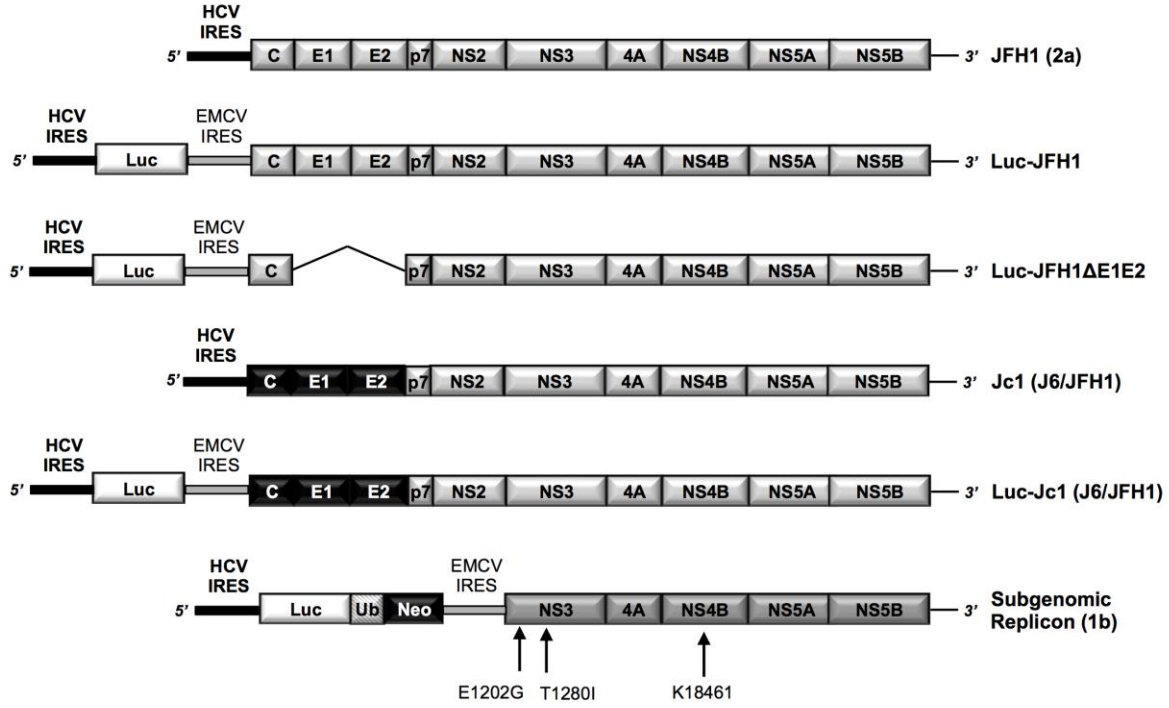
Knockdown of the GTPases Rab5 and Rab11 does not alter the inhibitory effect of LF-apoE3 on HCV replication. (A-B) HCV replicating cells were reverse-transfected with siRNA targeting Rab5 (A) and Rab11 (B) respectively. After 48h, cells were treated with LF-apoE3. Viral replication (luciferase activity) was quantified 48h post-treatment. (A, B) Silencing efficacy was assessed by WB. Results are expressed as % luciferase activity relative to cells Rab5- or Rab11-silenced HCV replicating cells treated with PBS (PBS, set at 100%). Means ±SD from two independent experiments done in triplicate are shown. . *$p < 0.01$; ** $p < 0.001$

Supplementary figure S5. Analysis of ABCG1 expression by RT-qPCR 72h after RNA silencing (A), or 8h after T0901317 treatment (B). Means ±SD from five (A) and two (B) independent experiments done in triplicate are shown. . ** $p < 0.001$

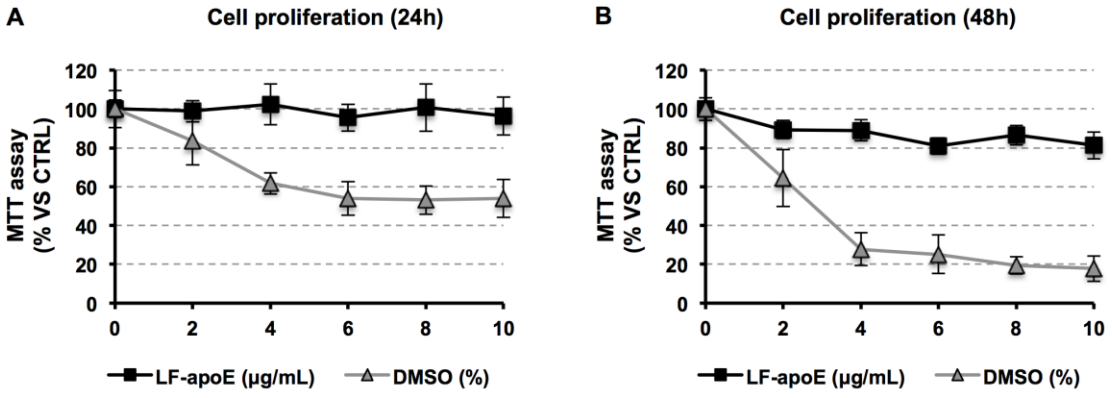
REFERENCE

1. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 1983 ; 65 :55-63.

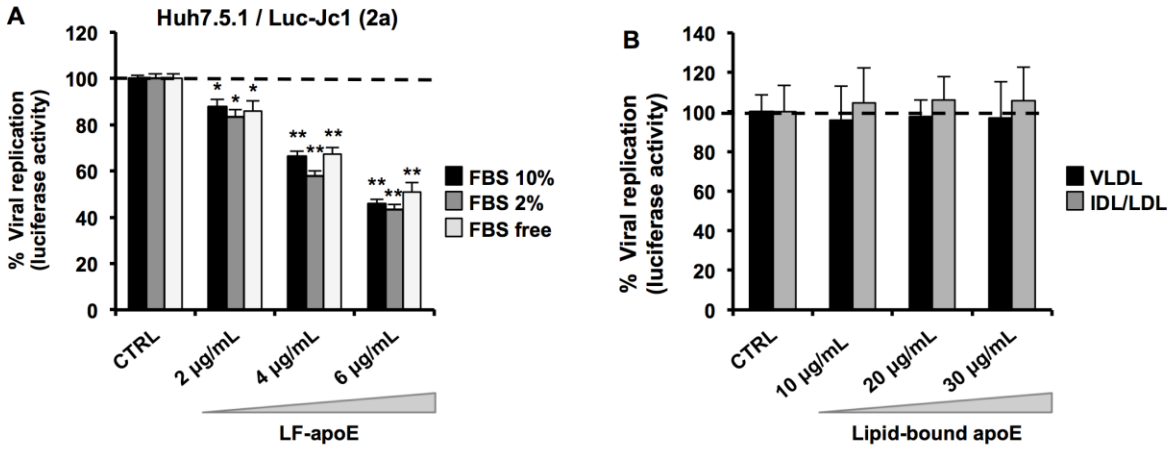
Supplementary Figures



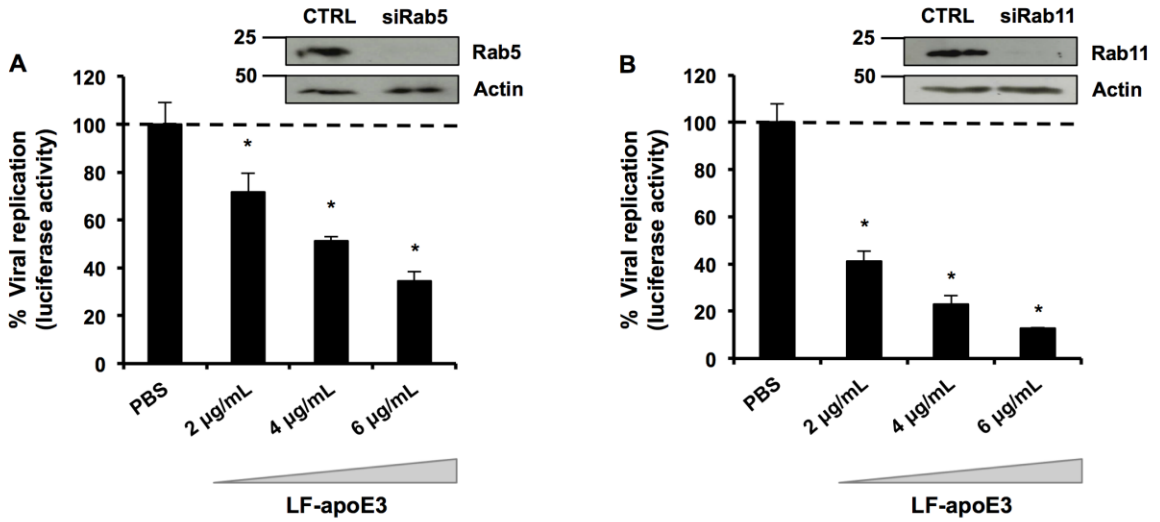
Supplementary figure 1: Schematic representation of viral genomes used in this study



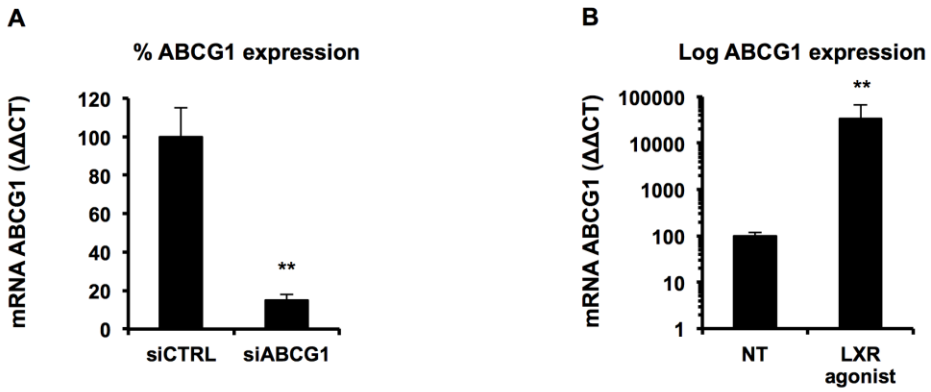
Supplementary figure 2: LF-apoE3 does not affect Huh7.5.1 cell proliferation



Supplementary figure 3: LF-apoE3 impairs HCV replication independently of serum components



Supplementary figure 4: Blocking apoE recycling does not impact LF-apoE3 's effect on HCV replication



Supplementary figure 5: Quantification of ABCG1 mRNA by RT-qPCR