



## **Claudin-1, miR-122 and apolipoprotein E transductions improve the permissivity of SNU-182, SNU-398 and SNU-449 hepatoma cells to hepatitis C virus**

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**Claudin-1, miR-122 and Apolipoprotein E  
transduction improves the permissivity of SNU-182,  
SNU-398 and SNU-449 Hepatoma cells to Hepatitis  
C Virus**

Carole Fournier<sup>1</sup>, Thomas Walter Hoffmann<sup>1</sup>, Virginie Morel<sup>1</sup>, Véronique Descamps<sup>1</sup>,  
Jean Dubuisson<sup>2</sup>, Etienne Brochot<sup>1</sup>, Catherine Francois<sup>1</sup>, Gilles Duverlie<sup>1</sup>, Sandrine Castelain<sup>1</sup>  
and Francois Helle<sup>1,\*</sup>

Author affiliations :

<sup>1</sup> EA4294, Laboratoire de Virologie, Centre Universitaire de Recherche en Santé, Centre  
Hospitalier Universitaire et Université de Picardie Jules Verne, 80054 Amiens, France.

<sup>2</sup> Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 8204 - CIIL  
- Centre d'Infection et d'Immunité de Lille, F-59000 Lille, France

\*Correspondence :

Dr. F. Helle, Laboratoire de Virologie EA4294, Centre Universitaire de Recherche en Santé,  
Centre Hospitalier Universitaire et Université de Picardie Jules Verne, 80054 Amiens, France.

Phone : (+33)-3-22-82-53-51

E-mail : francois.helle@u-picardie.fr

Running title : Permissivity of SNU cells to HCV

## Summary

Hepatitis C virus (HCV) is a human hepatotropic virus but many hepatoma cell lines are not permissive to this virus. In a previous study, we observed that SNU-182, SNU-398 and SNU-449 hepatoma cell lines were non-permissive to HCV. In order to understand the non-permissivity, we evaluated the ability of each cell line to support the different steps of HCV life cycle (entry, replication and production of infectious particles). Using retroviral pseudoparticles pseudotyped with HCV envelope proteins and recombinant HCV produced in cell culture, we observed that low level or absence of claudin-1 expression limited the viral entry process in SNU-182 and SNU-398 cells, respectively. Our results also showed that supplementation of the three cell lines with miR-122 partly restored the replication of a JFH1 HCV replicon. Finally, we observed that expression of apolipoprotein E was very low or undetectable in the three cell lines and that its ectopic expression permits the production of infectious viral particles in SNU-182 and SNU-398 cells but not in SNU-449 cells. Nevertheless, the supplementation of SNU-182, SNU-398 and SNU-449 cells with claudin-1, miR-122 and apolipoprotein E was not sufficient to render these cells as permissive as HuH-7 cells. Thus, these cell lines could serve as cell culture models for functional studies on the role of claudin-1, miR-122 and apolipoprotein E in HCV life cycle but also for the identification of new restriction and/or dependency host factors essential for HCV infection.

**Keywords :** ApoE, CLDN1, Hepatitis C virus, miR-122, restriction and dependency factors.

**Abbreviations :** HCV, Hepatitis C virus; HCVpp, retroviral particles pseudotyped with HCV envelope proteins; HCVcc, recombinant HCV produced in cell culture; SR-BI, Scavenger receptor class B type I; CLDN1, claudin-1; OCLN, occludin; NPC1L1, Niemann–Pick C1-like 1; ApoE, Apolipoprotein E.

## 1 | INTRODUCTION

Hepatitis C virus (HCV) is an important human pathogen. Worldwide, over 170 million people are chronically infected. Furthermore, 75% to 80% of HCV-infected patients develop chronic infection, which may lead to cirrhosis and, ultimately, hepatocellular carcinoma. HCV is a small enveloped positive-strand RNA virus of the *Flaviviridae* family, classified in the Hepacivirus genus (1).

The study of HCV infection has long been limited by the absence of complete *in vitro* cell culture system. However, several alternative tools were developed in the two past decades to understand the HCV life cycle. First, the establishment of subgenomic replicons that autonomously amplify in cultured human hepatoma cells allowed to understand the mechanisms of HCV genome replication (2). Then, the generation of infectious retroviral pseudotypes displaying functional HCV glycoproteins (HCVpp) allowed for the study of HCV entry (3, 4). The cloning of the genotype 2a JFH1 strain finally paved the way for the production of infectious HCV virions (HCVcc) and the investigation of the full viral cycle in human liver cancer-derived HuH-7 cells (5, 6). Thanks to these models, substantial progress has been made in the identification of host factors involved in HCV propagation. Among them, CD81, the scavenger receptor class B type I (SR-BI), claudin-1 (CLDN1) occludin (OCLN) and NPC1L1 were identified as important HCV entry factors (7). The liver-specific microRNA miR-122 has been shown to be one of the most important host factors for HCV replication (8). Furthermore, components of the very low density lipoprotein biosynthesis pathway such as apolipoprotein E (ApoE) have been shown to be important for the assembly and release of infectious viral particles (9-12).

The use of non-permissive cells is an interesting tool to identify new host restriction or dependency factors for viral permissivity. In a previous study, we reported that the SNU-182, SNU-398 and SNU-449 hepatocarcinoma cell lines were not able to support HCV infection

(13). In this work, we evaluated the ability of each cell line to support specific steps of HCV lifecycle (i.e. entry, replication and production of infectious particles) and tried to identify the host factors that prevent these cell lines from being infected by this virus.

## **2 | MATERIALS AND METHODS**

### **2.1 | Cell culture**

SNU-182 (CRL-2235), SNU-398 (CRL-2233) and SNU-449 (CRL-2234) cell lines (14) were obtained from ATCC. We also used the HuH-7 (RCB1366) (15) and HEK293T (CRL-11268) cell lines as control cells. All cell lines were cultured in Glutamax<sup>®</sup>-supplemented DMEM containing 10% fetal bovine serum.

### **2.2 | HCV Virus**

We used two recombinant viruses derived from the JFH1 strain and expressing a *Renilla* luciferase reporter gene, the JFH1-CS-A4-RLuc/TM construct (13) and the JFH1-CS-A4-RLuc/QM construct which contain the additional mutation I414T in E2, as compared to the JFH1-CS-A4-RLuc/TM strain.

### **2.3 | HCVcc and HCVpp infection**

HuH-7, SNU-182, SNU-398 and SNU-449 cells were inoculated for 3 hours with JFH1-derived constructs expressing the luciferase reporter gene. After inoculation, the medium was changed and cells were cultured for 72 hours. HCV infection was assessed by measuring the luciferase activity with a Berthold CentroXS<sup>3</sup> LB 960 luminometer. HCV entry in HuH-7, SNU-182, SNU-398, SNU-449 and 293T cells was evaluated using retroviral particles expressing firefly luciferase and pseudotyped with HCV JFH1 E1/E2 glycoproteins (HCVpp) (3). Non-infected cells or cells inoculated with particles lacking envelope protein ( $\Delta$ env) were

used as negative controls. Inoculation were performed during 3 hours and luciferase were measured 48 hours later.

#### **2.4 | Quantification of miR-122 expression**

The expression of miR-122-5p (assay ID: 002245) was quantified by RT-qPCR using the TaqMan® Small RNA Assays (Life Technologies), after extraction using the *mirVana*™ miRNA Isolation Kit (Life Technologies), according to the manufacturer's instructions. The small nuclear RNA RNU6B (assay ID: 001093) was used as endogenous control. The  $\Delta\Delta C_t$  method was used with HuH-7 cells as calibrator.

**2.5 | Flow cytometry.** The CD81 cell surface expression was assessed by flow cytometry. Cells were trypsinized, centrifuged, resuspended in PBS and stained with isotype control or FITC-coupled anti-CD81 mouse monoclonal antibody (JS-81, BD Pharmingen). Samples were analyzed on a Navios flow cytometer (Beckman Coulter).

**2.6 | CLDN1, ApoE and miR-122 transductions.** Lentiviral pseudoparticles were generated by co-transfection of 293T cells with plasmids encoding HIV gag-pol, the vesicular stomatitis virus envelope protein G (VSV-G) and the proteins of interest (pWPI-ApoE-BLR, kindly provided by T Pietschmann; pTRIP-Cerulean-CLDN1, kindly provided CM Rice). Cells were transduced by overnight incubation with lentiviral pseudoparticles at 37°C. Cells were also transduced with miR-122-expressing lentiviral particles (miRIDIAN shMIMIC Lentiviral microRNA, Thermo Scientific) to stably express miR-122 or the non-silencing control lentiviral particles (miRIDIAN shMIMIC Lentiviral microRNA, Thermo Scientific), as negative control.

**2.7 | Western blot analysis.** Cells were lysed with RIPA buffer. Protein content of cell lysates was determined using the BCA protein assay kit (Pierce). The proteins were precipitated, separated by SDS-PAGE and transferred to Hybond-ECL nitrocellulose membranes (GE Healthcare). Proteins were detected with specific antibodies diluted 1:1,000 (polyclonal anti-SR-B1 (Santa Cruz, ref sc-67098), polyclonal anti-Occludin (Invitrogen), polyclonal anti-Claudin 1 (Invitrogen), polyclonal anti-NPC1L1 (Cell Signaling), polyclonal anti-ApoE (AB947 Millipore) and monoclonal anti-actin (C4, Millipore) and secondary antibodies conjugated to peroxidase (Sigma-Aldrich). The immune complexes were visualized by enhanced chemiluminescence detection (ECL; GE Healthcare) as recommended by the manufacturer.

**2.8 | Quantification of HCV core protein and ApoE.** Intracellular or secreted HCV core antigen was quantified by a fully automated chemiluminescent microparticle immunoassay (Architect HCVA<sub>g</sub>; Abbott, Germany), according to the manufacturer's instructions. Concentration of human ApoE in the cell culture medium and lysates were determined by a specific ELISA assay, according to the manufacturer's instructions (Abcam).

**2.9 | Statistical analysis.** Mann-Whitney test was used to compare the results obtained with the different cell lines to that obtained with HuH-7 cells. Values of  $p < 0.05$  and  $< 0.01$  were considered as thresholds for significance.

## **3 | RESULTS**

**3.1 | SNU-182, SNU-398 and SNU-449 cells are not permissive to HCV entry and replication**

In a previous study, we used the RFP-NLS-IPS HCV infection reporter system (16) and observed that SNU-182, SNU-398 and SNU-449 cells were not permissive to HCV infection (13). This result indicated that the entry step and/or the replication step could not be achieved in these cells. In order to determine which step was affected, we first assessed HCV entry using HCVpp and pseudoparticles lacking envelope ( $\Delta$ Envpp) or non-infected cells as negative controls. As shown in Figure 1A, we observed that SNU-182 and SNU-449 cells enable low levels of HCV entry as compared to HuH-7 cells ( $2.8 \times 10^3$ ,  $2.0 \times 10^4$  and  $4.2 \times 10^5$  RLU, respectively) while the results obtained with SNU-398 were similar to that of the negative controls ( $2.2 \times 10^2$  RLU).

We also assessed HCV RNA replication after electroporation of the different cell lines with a JFH1 replicon expressing a luciferase reporter gene. Similar levels of electroporation were obtained for each cell line, as measured 4 hours after electroporation (1.5, 0.8, 0.6 and  $6.9 \times 10^5$  RLU for HuH-7, SNU-182, SNU-398 and SNU-449, respectively (Figure 1B)). In contrast, while HuH-7 cells achieved high levels of HCV replication ( $2.5 \times 10^7$  RLU at 72 h) the replication levels reached at 72 h in SNU-182, SNU-398 and SNU-449 cells were similar to that of the background measured in mock electroporated cells ( $1.0$ ,  $0.9$  and  $4.2 \times 10^3$  RLU at 72 h). Altogether, these results suggested that both the entry and replication steps of HCV life cycle are blocked in SNU-182, SNU-398 and SNU-449 cells.

### **3.2 | Stable expression of miR-122 partly restored HCV RNA replication in SNU-182, SNU-398 and SNU-449 cells**

Deficiency in miR-122 expression has been shown to be responsible for the absence of HCV RNA replication in several cell lines (17-20). For this reason, we assessed the expression of this miRNA by RT-qPCR in SNU-182, SNU-398 and SNU-449 cells. As shown in Figure 2A, we observed that these three cell lines expressed very low level of miR-122 as



compared to HuH-7 cells, at least 1000-fold lower. To investigate whether expression of miR-122 could restore HCV RNA replication in these cells, we transduced the miRNA with the help of lentiviruses. Transduction of 293T cells were used as positive control. While transduction of a control miRNA (miR-ctrl) had no effect on miR-122 expression, we observed that SNU-182, SNU-398, SNU-449 and 293T cells transduced with miR-122 express similar level of miR-122 as HuH-7 cells (Figure 2A). As previously demonstrated, we confirmed that transduction of miR-122 in 293T cells permit to reach similar level of HCV RNA replication as in HuH-7 cells ( $2.2 \cdot 10^7$  and  $4.3 \cdot 10^7$  RLU at 72 h, respectively; Figure 2B). In contrast, although HCV RNA replication was improved, only low levels were achieved after miR-122 transduction of SNU-182, SNU-398 and SNU-449 cells, ( $8.5 \cdot 10^5$ ,  $2.4 \cdot 10^5$  and  $1.1 \cdot 10^5$  RLU at 72 h, respectively; Figure 2B). This low level of HCV RNA replication in SNU-182, SNU-398 and SNU-449 transduced cells was confirmed by the detection of intracellular Core protein (Figure 2C). Thus, these results indicate that the expression of miR-122 in SNU-182, SNU-398 and SNU-449 improves the replication of HCV RNA but is not sufficient to achieve high level of replication.

Recently, it has also been shown that SEC14L2 enables pan-genotype HCV replication in cell culture (21). We thus investigated whether transduction of SEC14L2 in SNU-182, SNU-398 and SNU-449 cells could improve HCV replication but we did not observe any effect (data not shown). However, this result was predictable since Saeed *et al.* also mentioned that replication of the JFH-1 strain was not enhanced by SEC14L2. We also wondered whether induction of the innate immune response could be responsible for the low replication level, however we did not detect any induction of Interferon- $\beta$  and Interferon- $\lambda$  mRNA expression after infection (data not shown). This was corroborated by the fact that the use of pyridone-6, an inhibitor of the Janus Kinases involved in the Interferon-mediated signaling, did not increase the replication level (data not shown).

Finally, since low levels of replication were achieved in SNU-182, SNU-398 and SNU-449 cells transduced with miR-122, we wondered whether they were able to assemble and secrete progeny virions. However, we did not detect any infectious particle production (Figure 2D) and levels of extracellular Core protein remained very low or undetectable (Figure 2C).

### **3.3 | CLDN1 transduction improves HCV entry in SNU-182-miR-122 and SNU-398-miR-122 cells**

To understand HCV entry defect in SNU-182, SNU-398 and SNU-449 cells, we evaluated the expression level of several cellular entry factors. Flow cytometry analysis revealed that CD81 is correctly expressed at the surface of each cell line (Figure 3A). In addition, we observed by western blot using specific antibodies that the three cell lines expressed high levels of SR-BI, OCLN and NPC1L1 (Figure 3B). In contrast, CLDN1 was only very slightly detected in SNU-182 cells and undetectable in SNU-398 cells.

Using SNU-182, SNU-398 and SNU-449 cells transduced with miR-122, we investigated whether stable expression of exogenous CLDN1 could improve HCV entry. These cells were transduced with lentiviruses expressing Cerulean-CLDN1 and 293T cells were used as control. The efficiency of the transduction was checked by western blot (Figure 4A) and the effect on HCV entry was evaluated with the help of HCVpp and HCVcc (Figure 4B). These experiments showed that exogenous CLDN1 expression slightly improves HCVpp entry in SNU-182-miR-122 and SNU-398-miR-122 cells as compared to the increase observed in 293T cells (8.3-, 5.7- and 64.2-fold increase respectively, as compared to cells transduced with a control lentivirus). The improvement observed in HCVcc entry was lower, probably because of the low RNA replication level in these cells, as described above. In

addition, our results suggest that Cerulean-CLDN1 transduction did not have any effect on HCV entry in SNU-449-miR-122 cells.

### **3.4 | ApoE transduction improves infectious virus production in SNU-182-miR-122-CLDN1 and SNU-398-miR-122-CLDN1 cells**

As shown in Figure 2B and 2D, whereas low levels of HCV RNA replication were achieved in miR-122 transduced SNU-182, SNU-398 and SNU-449 cells, we did not detect any production of progeny virions. Since ApoE is known to be essential for assembly and secretion of new virions, we assessed the expression level of this protein in these cells. Western blot analysis and ELISA assay suggested that ApoE was not expressed in SNU-398-miR-122-CLDN1 and SNU-449-miR-122 cells whereas it was marginally expressed and secreted by SNU-182-miR-122-CLDN1 cells. We thus tested whether stable expression of exogenous ApoE could help these cells to produce infectious virions. After transduction, ApoE was readily detectable in cell lysates and cell supernatants of SNU-398-miR-122-CLDN1 and SNU-449-miR-122 cells (Figure 5A and 5B). In contrast, while it was readily detected in the cell lysate of SNU-182-miR-122-CLDN1 cells, the concentration in cell supernatant was low, suggesting a potential defect in secretion. Interestingly, we observed that stable expression of ApoE in SNU-182-miR-122-CLDN1 and SNU-398-miR-122-CLDN1 cells gave rise to a production of progeny virions whereas it had no effect on viral production in SNU-449-miR-122 cells (Figure 5C). These results were confirmed by quantification of Core in the cell supernatant (Figure 5D).

## **4 | DISCUSSION**

In our previous study, we identified three human hepatocellular carcinoma cell lines established from the primary tumors of Korean patients, SNU-182, SNU-398 and SNU-449,

that are not permissive to HCV (13). For each cell line, we identified the different steps that were compromised and tried to decipher the mechanisms of the impairments (cf summary Table 1).

We first observed that defect in CLDN1 expression was responsible for the inefficiency of the entry step in SNU-182 and SNU-398. We also demonstrated that its ectopic expression render these cells permissive to this step. CLDN1 was identified as an HCV entry factor thanks to a cyclic lentivirus based repackaging screen of a complementary DNA library, derived from the highly HCV-permissive hepatocarcinoma Huh-7.5 cell line, for genes that render the 293T cell line permissive to HCVpp (22). For this reason, 293T cells are widely used to perform functional studies on the role of CLDN1 in HCV entry. It has also been shown that CLDN1 expression alone conferred limited permissiveness for HCV infection in these cells and that co-expression of CD81, OCLN and SR-BI enhance HCV entry to a level higher than that in Huh7.5.1 cells (20). However, it has to be kept in mind that many clones of 293T cells exist which may explain some discrepancies between studies. Other models such as H1H or HuH6 cells have been used (23, 24) and in this study we identified SNU-182 and SNU-398 cells as alternative cell culture models, expressing high levels of CD81, OCLN, SR-BI and NPC1L1, to perform functional studies of the role of CLDN1 in HCV entry.

MiR-122 is an abundant, liver-specific miRNA that is an unusual host factor for HCV. This miRNA binds HCV RNA and slows decay of the viral genome through protection from 5' exonuclease activity of the host mRNA decay machinery (25). Many studies demonstrated that supplementation of several non-permissive cell lines with miR-122 render them permissive for transient replication of HCV RNA (17-20, 26-28). In this study, we observed that miR-122 is expressed at a low level in SNU-182, SNU-398 and SNU-449 cells. We also demonstrated that transduction of this miRNA in these cells improves HCV RNA replication.

However, in contrast to 293T transduced cells, viral replication in SNU-182, SNU-398 and SNU-449 cells remained very low even after transduction and this was not due to induction of innate immune response. It is thus likely that other dependency host factors essential for the replication are lacking. Alternatively, this could be explained by the presence of restriction factors that decrease the efficiency of this step. Our results also suggest that transduction of ApoE permit the production of low level of HCV infectious particles in SNU-182 and SNU-398 cells transduced with miR-122 but not in SNU-449 cells. However, it is difficult to evaluate precisely the efficiency of this step because of the low level of replication.

Altogether, our results identified SNU-182, SNU-398 and SNU-449 cells as hepatocarcinoma cell lines that can be used as models for the study of the functional role of CLDN1, miR-122 and ApoE in the entry, replication and assembly step of HCV life cycle. These cell lines could also be used to identify new dependency/restriction host factors essential for the different step of the viral cycle, thanks to screening strategies, as described previously (21, 22, 29).

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## Conflicts of interest

The authors declare that there are no conflict of interest.

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## FIGURE LEGENDS

**Figure 1** HCV entry and replication in SNU-182, SNU-398 and SNU-449 cells. A. HuH-7, SNU-182, SNU-398 and SNU-449 cells were inoculated for 4 h with HCVpp or  $\Delta$ envpp to monitor HCV entry. Luciferase assays were performed 48 h post-infection. Results are expressed as Relative Light Units (RLU) and are reported as the means  $\pm$  S.D. of three independent experiments. B. JFH1 RNA encoding a luciferase reporter gene and deleted for E1E2 region was electroporated in HuH-7, SNU-182, SNU-398 and SNU-449 cells. RNA Replication was assessed by measuring luciferase activity at 4 and 72 h post-electroporation. Results are expressed as Relative Light Units (RLU) and are reported as the means  $\pm$  S.D. of three independent experiments. Differences were considered statistically significant if  $p < 0.05$  (\*) or  $p < 0.01$  (\*\*).

**Figure 2** Stable expression of miR-122 partly restored HCV RNA replication in SNU-182, SNU-398 and SNU-449 cells. A. The expression of miR-122 was determined by RT-qPCR in HuH-7, SNU-182, SNU-398, SNU-449 and 293T cells, before (NT for non-transduced) or after transduction with lentiviruses expressing a control miRNA (miR-ctrl) or miR-122. The  $\Delta\Delta$ Ct method was used for quantification, with the small nuclear RNA RNU6B as endogenous control and HuH-7 cells as calibrator. All results are representative of 3 independent experiments. B. SNU-182, SNU-398, SNU-449 and 293T cells, transduced with miR-ctrl or miR-122, were electroporated with JFH1 RNA encoding a luciferase reporter gene. The replication was assessed by measuring luciferase activities at 4, 24, 48 and 72h. Results are expressed as RLU and are reported as means  $\pm$  S.D. of three independent experiments. C. Core protein concentration was determined in the cell culture supernatants and the cell lysates recovered 72h post-electroporation. Results are reported as the means  $\pm$  S.D. of three

independent experiments (ND : not determined). D. Infectious particle production was evaluated after inoculation of naive HuH-7 cells with the supernatants recovered from electroporated cells. HuH-7 cells were inoculated during 4 h and luciferase activities were measured 72 h post-infection. Results are expressed as RLU and are reported as the means  $\pm$  S.D. of three independent experiments. Differences were considered statistically significant if  $p < 0.05$  (\*) or  $p < 0.01$  (\*\*).

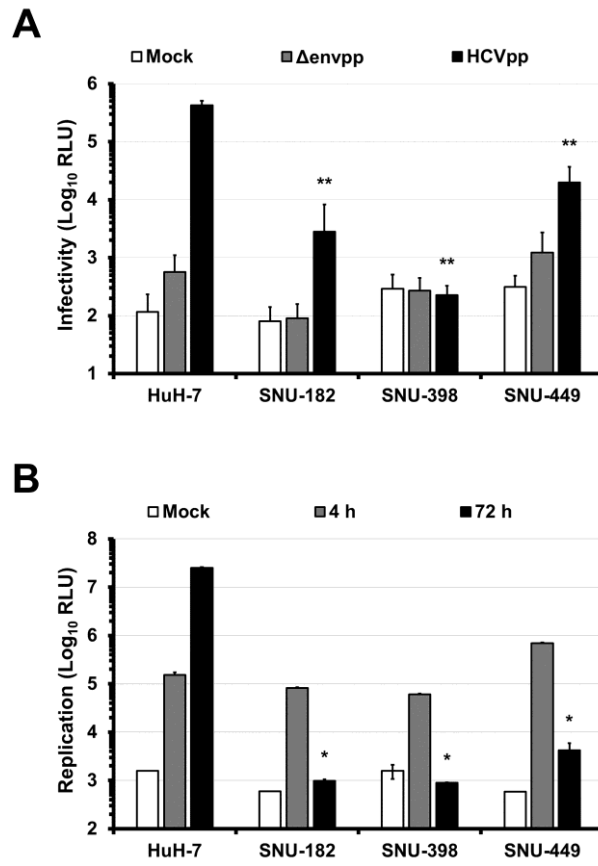
**Figure 3** Evaluation of HCV cell entry factor expression in SNU-182, SNU-398 and SNU-449 cells. A. Flow cytometry was performed to assess the expression of CD81 in SNU-182, SNU-398, and SNU-449, using FITC-coupled anti-CD81 antibody (grey) or isotype control (white). HuH-7 cells were used as control. B. SR-B1, CLDN1, OCLN and NPC1L1 expression in each cell line was evaluated by western blot.  $\beta$ -actin served as a loading control. The molecular weight (MW) of each protein is indicated on the left.

**Figure 4** Ectopic expression of CLDN1 in SNU-182-miR-122 and SNU-398-miR-122 cells improves HCV entry. A. SNU-182-miR-122, SNU-398-miR-122, SNU-449-miR-122 and 293T cells were transduced with lentiviruses expressing Cerulean-CLDN1. Empty lentiviruses were used as negative control. Cerulean-CLDN1 expression was evaluated by western blot.  $\beta$ -actin served as a loading control. The molecular weight (MW) of each protein is indicated on the left. B. HCV entry in each cell line was evaluated after inoculation with HCVpp or HCVcc for 4 h. 293T cells were used as control. Infection was determined by measuring luciferase activities 72h post-infection. Results are expressed as RLU and are reported as the means  $\pm$  S.D. of three independent experiments. Differences were considered statistically significant if  $p < 0.05$  (\*) or  $p < 0.01$  (\*\*).

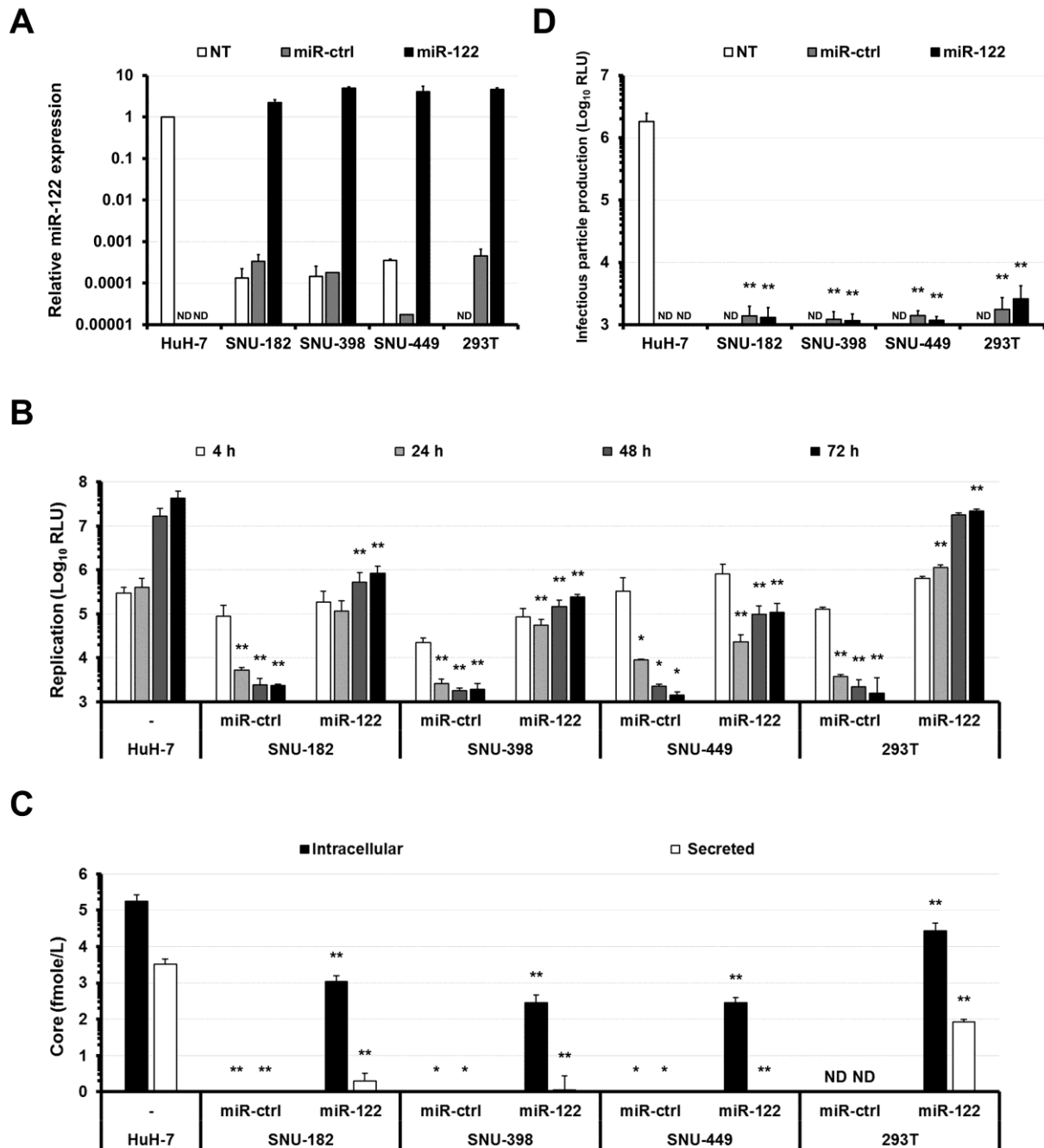
**Figure 5** Ectopic expression of ApoE enables HCVcc production in the SNU182-miR-122-CLDN-1 and SNU398-miR-122-CLDN-1 cells. A. SNU-182-miR-122-CLDN1, SNU-398-miR-122-CLDN1 cells and SNU-449-miR-122 cells were transduced with lentiviruses expressing ApoE. HuH-7 and 293T-miR122-CLDN1 cells were used as control. Non-transduced cells were used as negative control. ApoE expression was evaluated by western blot.  $\beta$ -actin served as a loading control. The molecular weight (MW) of each protein is indicated on the left. B. The concentration of ApoE secreted in the supernatant of each cell line after 72 h was evaluated by Elisa assay. Results are reported as the means  $\pm$  S.D. of two independent experiments. C. The different cell lines were electroporated with JFH1 RNA encoding a luciferase reporter gene and the supernatants were recovered 72 h after electroporation. Infectious particle production was evaluated by inoculating naive HuH-7 cells with these supernatants for 4 h and measuring luciferase activities 72 h post-infection. Results are expressed as RLU and are reported as the means  $\pm$  S.D. of three independent experiments. D. Core protein concentration in the cell culture supernatants and the cell lysates was determined 72h post-electroporation. Results are reported as the means  $\pm$  S.D. of at least two independent experiments. Differences were considered statistically significant if  $p < 0.05$  (\*) or  $p < 0.01$  (\*\*).

**TABLE 1** Features of the cell lines used in this study

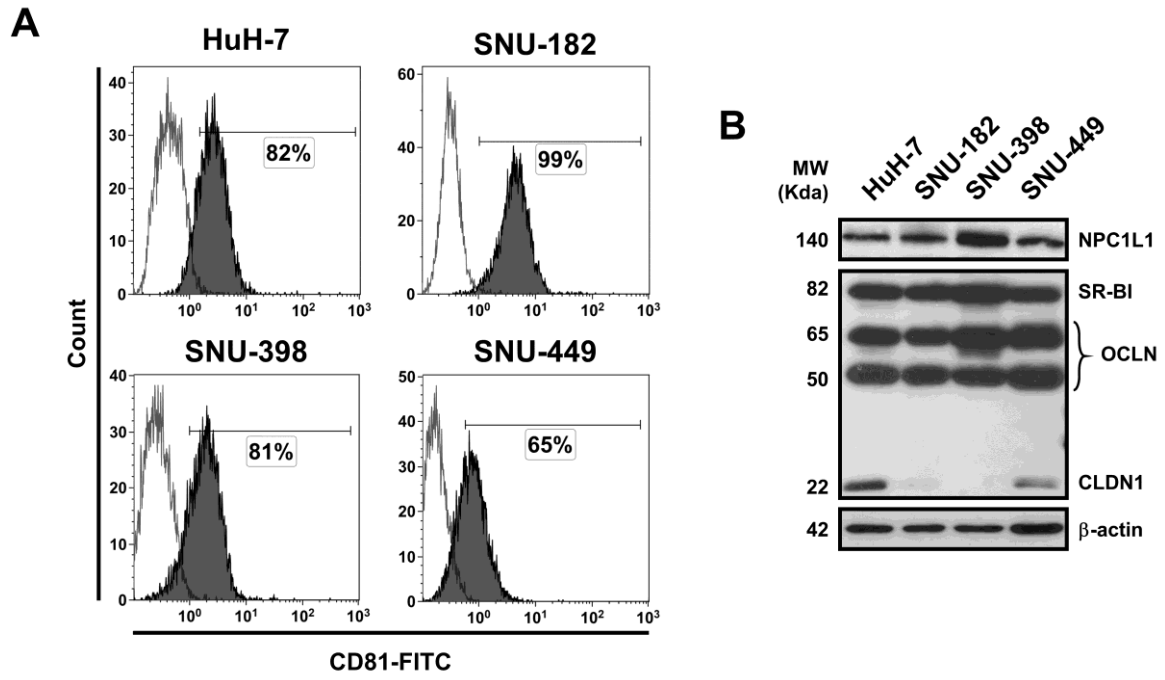
Cell line	Entry	Replication	Assembly	CD81	SR-BI	CLDN1	OCLN	NPC1L1	mir-122	ApoE
HuH-7	++	++	++	++	++	++	++	++	++	++
SNU-182	+/-	-	-	++	++	+/-	++	++	-	+
SNU-182-miR122	+/-	+	-	++	++	+/-	++	++	++	+
SNU-182-miR-122-CLDN1	+	+	-	++	++	++	++	++	++	+
SNU-182-miR-122-CLDN1-ApoE	+	+	+	++	++	++	++	++	++	++
SNU-398	-	-	-	++	++	-	++	++	-	-
SNU-398-miR122	-	+	-	++	++	-	++	++	++	-
SNU-398-miR-122-CLDN1	+	+	-	++	++	++	++	++	++	-
SNU-398-miR-122-CLDN1-ApoE	+	+	+	++	++	++	++	++	++	++
SNU-449	+	-	-	++	++	+	++	++	-	-
SNU-449-miR122	+	+	-	++	++	+	++	++	++	-
SNU-449-miR-122-CLDN1	+	+	-	++	++	++	++	++	++	-
SNU-449-miR-122-ApoE	+	+	-	++	++	+	++	++	++	++

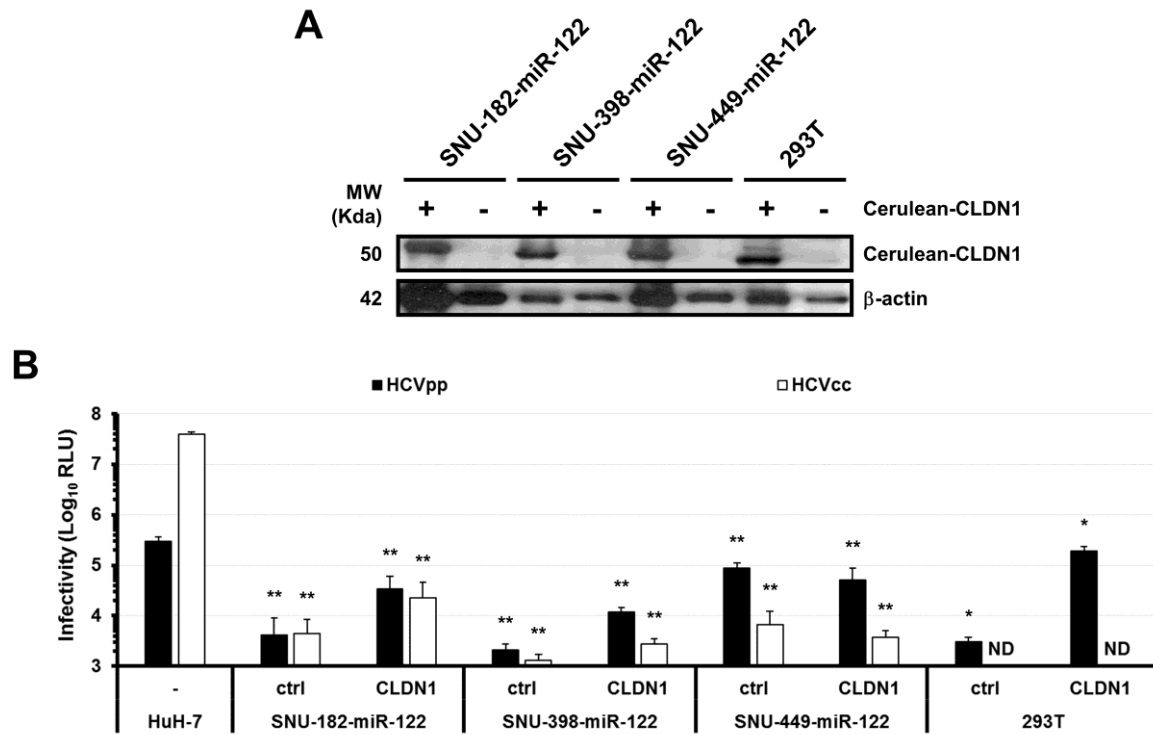


**FIG 1**



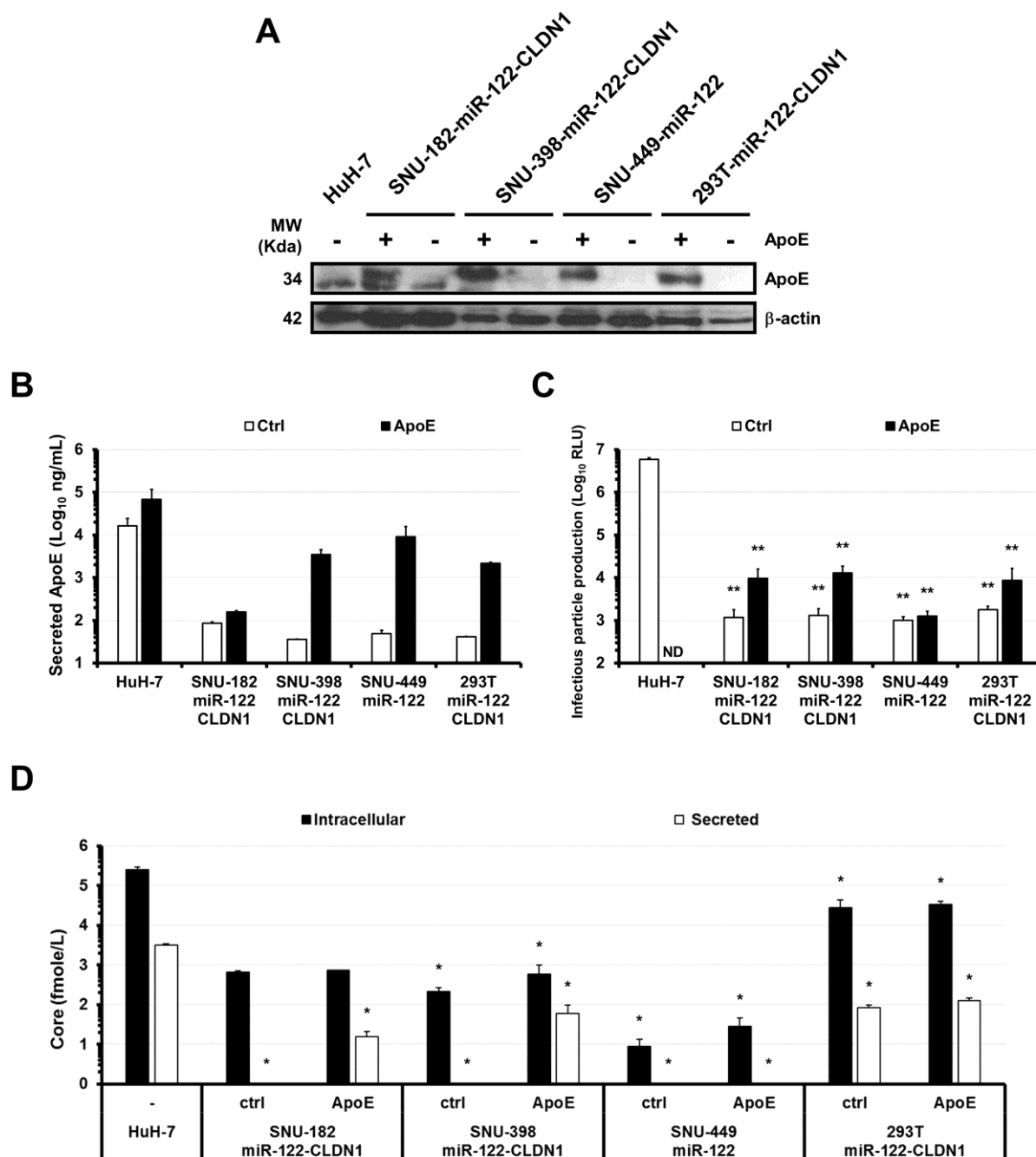
**FIG 2**





**FIG 4**




**FIG 5**