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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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24 **Summary**

25 Hepatitis C virus (HCV) is a human hepatotropic virus but many hepatoma cell lines are not
26 permissive to this virus. In a previous study, we observed that SNU-182, SNU-398 and SNU-
27 449 hepatoma cell lines were non-permissive to HCV. In order to understand the non-
28 permissivity, we evaluated the ability of each cell line to support the different steps of HCV
29 life cycle (entry, replication and production of infectious particles). Using retroviral
30 pseudoparticles pseudotyped with HCV envelope proteins and recombinant HCV produced in
31 cell culture, we observed that low level or absence of claudin-1 expression limited the viral
32 entry process in SNU-182 and SNU-398 cells, respectively. Our results also showed that
33 supplementation of the three cell lines with miR-122 partly restored the replication of a JFH1
34 HCV replicon. Finally, we observed that expression of apolipoprotein E was very low or
35 undetectable in the three cell lines and that its ectopic expression permits the production of
36 infectious viral particles in SNU-182 and SNU-398 cells but not in SNU-449 cells.
37 Nevertheless, the supplementation of SNU-182, SNU-398 and SNU-449 cells with claudin-1,
38 miR-122 and apolipoprotein E was not sufficient to render these cells as permissive as HuH-7
39 cells. Thus, these cell lines could serve as cell culture models for functional studies on the role
40 of claudin-1, miR-122 and apolipoprotein E in HCV life cycle but also for the identification
41 of new restriction and/or dependency host factors essential for HCV infection.

42

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

44

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

49

50 1 | INTRODUCTION

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

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

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

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

78

79 **2 | MATERIALS AND METHODS**

80 **2.1 | Cell culture**

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

85

86 **2.2 | HCV Virus**

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

91

92 **2.3 | HCVcc and HCVpp infection**

93 HuH-7, SNU-182, SNU-398 and SNU-449 cells were inoculated for 3 hours with JFH1-
94 derived constructs expressing the luciferase reporter gene. After inoculation, the medium was
95 changed and cells were cultured for 72 hours. HCV infection was assessed by measuring the
96 luciferase activity with a Berthold CentroXS³ LB 960 luminometer. HCV entry in HuH-7,
97 SNU-182, SNU-398, SNU-449 and 293T cells was evaluated using retroviral particles
98 expressing firefly luciferase and pseudotyped with HCV JFH1 E1/E2 glycoproteins (HCVpp)
99 (3). Non-infected cells or cells inoculated with particles lacking envelope protein (Δ env) were

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

102

103 **2.4 | Quantification of miR-122 expression**

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

109

110 **2.5 | Flow cytometry.** The CD81 cell surface expression was assessed by flow cytometry.

111 Cells were trypsinized, centrifuged, resuspended in PBS and stained with isotype control or
112 FITC-coupled anti-CD81 mouse monoclonal antibody (JS-81, BD Pharmingen). Samples
113 were analyzed on a Navios flow cytometer (Beckman Coulter).

114

115 **2.6 | CLDN1, ApoE and miR-122 transductions.** Lentiviral pseudoparticles were generated
116 by co-transfection of 293T cells with plasmids encoding HIV gag-pol, the vesicular stomatitis
117 virus envelope protein G (VSV-G) and the proteins of interest (pWPI-ApoE-BLR, kindly
118 provided by T Pietschmann; pTRIP-Cerulean-CLDN1, kindly provided CM Rice). Cells were
119 transduced by overnight incubation with lentiviral pseudoparticles at 37°C. Cells were also
120 transduced with miR-122-expressing lentiviral particles (miRIDIAN shMIMIC Lentiviral
121 microRNA, Thermo Scientific) to stably express miR-122 or the non-silencing control
122 lentiviral particles (miRIDIAN shMIMIC Lentiviral microRNA, Thermo Scientific), as
123 negative control.

124

125 **2.7 | Western blot analysis.** Cells were lysed with RIPA buffer. Protein content of cell
126 lysates was determined using the BCA protein assay kit (Pierce). The proteins were
127 precipitated, separated by SDS-PAGE and transferred to Hybond-ECL nitrocellulose
128 membranes (GE Healthcare). Proteins were detected with specific antibodies diluted 1:1,000
129 (polyclonal anti-SR-B1 (Santa Cruz, ref sc-67098), polyclonal anti-Occludin (Invitrogen),
130 polyclonal anti-Claudin 1 (Invitrogen), polyclonal anti-NPC1L1 (Cell Signaling), polyclonal
131 anti-ApoE (AB947 Millipore) and monoclonal anti-actin (C4, Millipore) and secondary
132 antibodies conjugated to peroxidase (Sigma-Aldrich). The immune complexes were
133 visualized by enhanced chemiluminescence detection (ECL; GE Healthcare) as recommended
134 by the manufacturer.

135

136 **2.8 | Quantification of HCV core protein and ApoE.** Intracellular or secreted HCV core
137 antigen was quantified by a fully automated chemiluminescent microparticle immunoassay
138 (Architect HCVAg; Abbott, Germany), according to the manufacturer's instructions.
139 Concentration of human ApoE in the cell culture medium and lysates were determined by a
140 specific ELISA assay, according to the manufacturer's instructions (Abcam).

141

142 **2.9 | Statistical analysis.** Mann-Whitney test was used to compare the results obtained with
143 the different cell lines to that obtained with HuH-7 cells. Values of $p < 0.05$ and < 0.01 were
144 considered as thresholds for significance.

145

146 **3 | RESULTS**

147 **3.1 | SNU-182, SNU-398 and SNU-449 cells are not permissive to HCV entry and**
148 **replication**

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

158 We also assessed HCV RNA replication after electroporation of the different cell lines
159 with a JFH1 replicon expressing a luciferase reporter gene. Similar levels of electroporation
160 were obtained for each cell line, as measured 4 hours after electroporation (1.5, 0.8, 0.6 and
161 $6.9 \cdot 10^5$ RLU for HuH-7, SNU-182, SNU-398 and SNU-449, respectively (Figure 1B)). In
162 contrast, while HuH-7 cells achieved high levels of HCV replication ($2.5 \cdot 10^7$ RLU at 72 h)
163 the replication levels reached at 72 h in SNU-182, SNU-398 and SNU-449 cells were similar
164 to that of the background measured in mock electroporated cells (1.0 , 0.9 and $4.2 \cdot 10^3$ RLU at
165 72 h). Altogether, these results suggested that both the entry and replication steps of HCV life
166 cycle are blocked in SNU-182, SNU-398 and SNU-449 cells.

167

168 **3.2 | Stable expression of miR-122 partly restored HCV RNA replication in SNU-182,** 169 **SNU-398 and SNU-449 cells**

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

174 compared to HuH-7 cells, at least 1000-fold lower. To investigate whether expression of miR-
175 122 could restore HCV RNA replication in these cells, we transduced the miRNA with the
176 help of lentiviruses. Transduction of 293T cells were used as positive control. While
177 transduction of a control miRNA (miR-ctrl) had no effect on miR-122 expression, we
178 observed that SNU-182, SNU-398, SNU-449 and 293T cells transduced with miR-122
179 express similar level of miR-122 as HuH-7 cells (Figure 2A). As previously demonstrated, we
180 confirmed that transduction of miR-122 in 293T cells permit to reach similar level of HCV
181 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).
182 In contrast, although HCV RNA replication was improved, only low levels were achieved
183 after miR-122 transduction of SNU-182, SNU-398 and SNU-449 cells, ($8.5 \cdot 10^5$, $2.4 \cdot 10^5$ and
184 $1.1 \cdot 10^5$ RLU at 72 h, respectively; Figure 2B). This low level of HCV RNA replication in
185 SNU-182, SNU-398 and SNU-449 transduced cells was confirmed by the detection of
186 intracellular Core protein (Figure 2C). Thus, these results indicate that the expression of miR-
187 122 in SNU-182, SNU-398 and SNU-449 improves the replication of HCV RNA but is not
188 sufficient to achieve high level of replication.

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

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

204

205 **3.3 | CLDN1 transduction improves HCV entry in SNU-182-miR-122 and SNU-398-** 206 **miR-122 cells**

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

213 Using SNU-182, SNU-398 and SNU-449 cells transduced with miR-122, we
214 investigated whether stable expression of exogenous CLDN1 could improve HCV entry.
215 These cells were transduced with lentiviruses expressing Cerulean-CLDN1 and 293T cells
216 were used as control. The efficiency of the transduction was checked by western blot (Figure
217 4A) and the effect on HCV entry was evaluated with the help of HCVpp and HCVcc (Figure
218 4B). These experiments showed that exogenous CLDN1 expression slightly improves HCVpp
219 entry in SNU-182-miR-122 and SNU-398-miR-122 cells as compared to the increase
220 observed in 293T cells (8.3-, 5.7- and 64.2-fold increase respectively, as compared to cells
221 transduced with a control lentivirus). The improvement observed in HCVcc entry was lower,
222 probably because of the low RNA replication level in these cells, as described above. In

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

225

226 **3.4 | ApoE transduction improves infectious virus production in SNU-182-miR-122- 227 CLDN1 and SNU-398-miR-122-CLDN1 cells**

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

244

245 **4 | DISCUSSION**

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

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

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

266 MiR-122 is an abundant, liver-specific miRNA that is an unusual host factor for HCV.
267 This miRNA binds HCV RNA and slows decay of the viral genome through protection from
268 5' exonuclease activity of the host mRNA decay machinery (25). Many studies demonstrated
269 that supplementation of several non-permissive cell lines with miR-122 render them
270 permissive for transient replication of HCV RNA (17-20, 26-28). In this study, we observed
271 that miR-122 is expressed at a low level in SNU-182, SNU-398 and SNU-449 cells. We also
272 demonstrated that transduction of this miRNA in these cells improves HCV RNA replication.

273 However, in contrast to 293T transduced cells, viral replication in SNU-182, SNU-398 and
274 SNU-449 cells remained very low even after transduction and this was not due to induction of
275 innate immune response. It is thus likely that other dependency host factors essential for the
276 replication are lacking. Alternatively, this could be explained by the presence of restriction
277 factors that decrease the efficiency of this step. Our results also suggest that transduction of
278 ApoE permit the production of low level of HCV infectious particles in SNU-182 and SNU-
279 398 cells transduced with miR-122 but not in SNU-449 cells. However, it is difficult to
280 evaluate precisely the efficiency of this step because of the low level of replication.

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

287

288

289

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293

294 **Conflicts of interest**

295 The authors declare that there are no conflict of interest.

296

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- 378
- 379
- 380

381 **FIGURE LEGENDS**

382

383 **Figure 1** HCV entry and replication in SNU-182, SNU-398 and SNU-449 cells. A. HuH-7,
384 SNU-182, SNU-398 and SNU-449 cells were inoculated for 4 h with HCVpp or Δ envpp to
385 monitor HCV entry. Luciferase assays were performed 48 h post-infection. Results are
386 expressed as Relative Light Units (RLU) and are reported as the means \pm S.D. of three
387 independent experiments. B. JFH1 RNA encoding a luciferase reporter gene and deleted for
388 E1E2 region was electroporated in HuH-7, SNU-182, SNU-398 and SNU-449 cells. RNA
389 Replication was assessed by measuring luciferase activity at 4 and 72 h post-electroporation.
390 Results are expressed as Relative Light Units (RLU) and are reported as the means \pm S.D. of
391 three independent experiments. Differences were considered statistically significant if
392 $p < 0.05$ (*) or $p < 0.01$ (**).

393

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

406 independent experiments (ND : not determined). D. Infectious particle production was
407 evaluated after inoculation of naive HuH-7 cells with the supernatants recovered from
408 electroporated cells. HuH-7 cells were inoculated during 4 h and luciferase activities were
409 measured 72 h post-infection. Results are expressed as RLU and are reported as the means \pm
410 S.D. of three independent experiments. Differences were considered statistically significant if
411 $p < 0.05$ (*) or $p < 0.01$ (**).

412

413 **Figure 3** Evaluation of HCV cell entry factor expression in SNU-182, SNU-398 and SNU-
414 449 cells. A. Flow cytometry was performed to assess the expression of CD81 in SNU-182,
415 SNU-398, and SNU-449, using FITC-coupled anti-CD81 antibody (grey) or isotype control
416 (white). HuH-7 cells were used as control. B. SR-B1, CLDN1, OCLN and NPC1L1
417 expression in each cell line was evaluated by western blot. β -actin served as a loading control.
418 The molecular weight (MW) of each protein is indicated on the left.

419

420 **Figure 4** Ectopic expression of CLDN1 in SNU-182-miR-122 and SNU-398-miR-122 cells
421 improves HCV entry. A. SNU-182-miR-122, SNU-398-miR-122, SNU-449-miR-122 and
422 293T cells were transduced with lentiviruses expressing Cerulean-CLDN1. Empty
423 lentiviruses were used as negative control. Cerulean-CLDN1 expression was evaluated by
424 western blot. β -actin served as a loading control. The molecular weight (MW) of each protein
425 is indicated on the left. B. HCV entry in each cell line was evaluated after inoculation with
426 HCVpp or HCVcc for 4 h. 293T cells were used as control. Infection was determined by
427 measuring luciferase activities 72h post-infection. Results are expressed as RLU and are
428 reported as the means \pm S.D. of three independent experiments. Differences were considered
429 statistically significant if $p < 0.05$ (*) or $p < 0.01$ (**).

430

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

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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	+	+	-	++	++	+	++	++	++	++

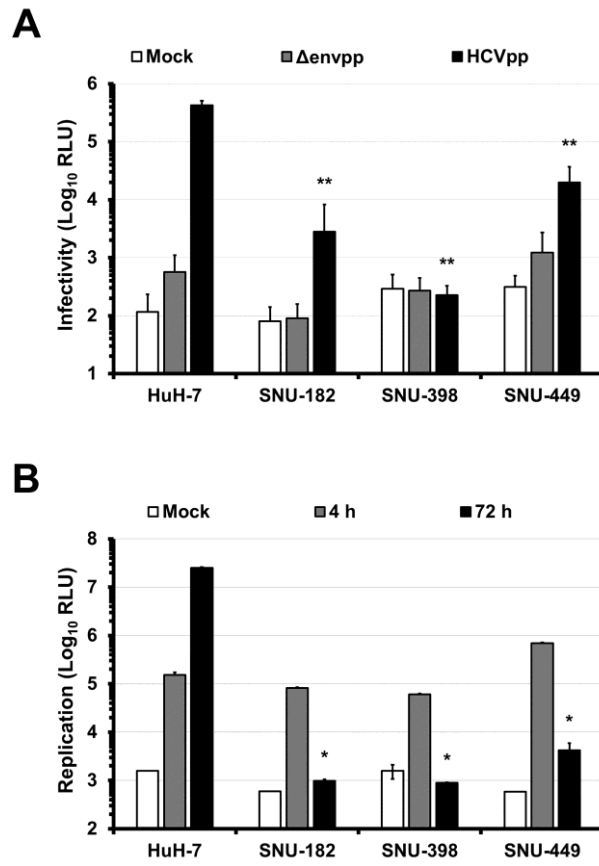


FIG 1

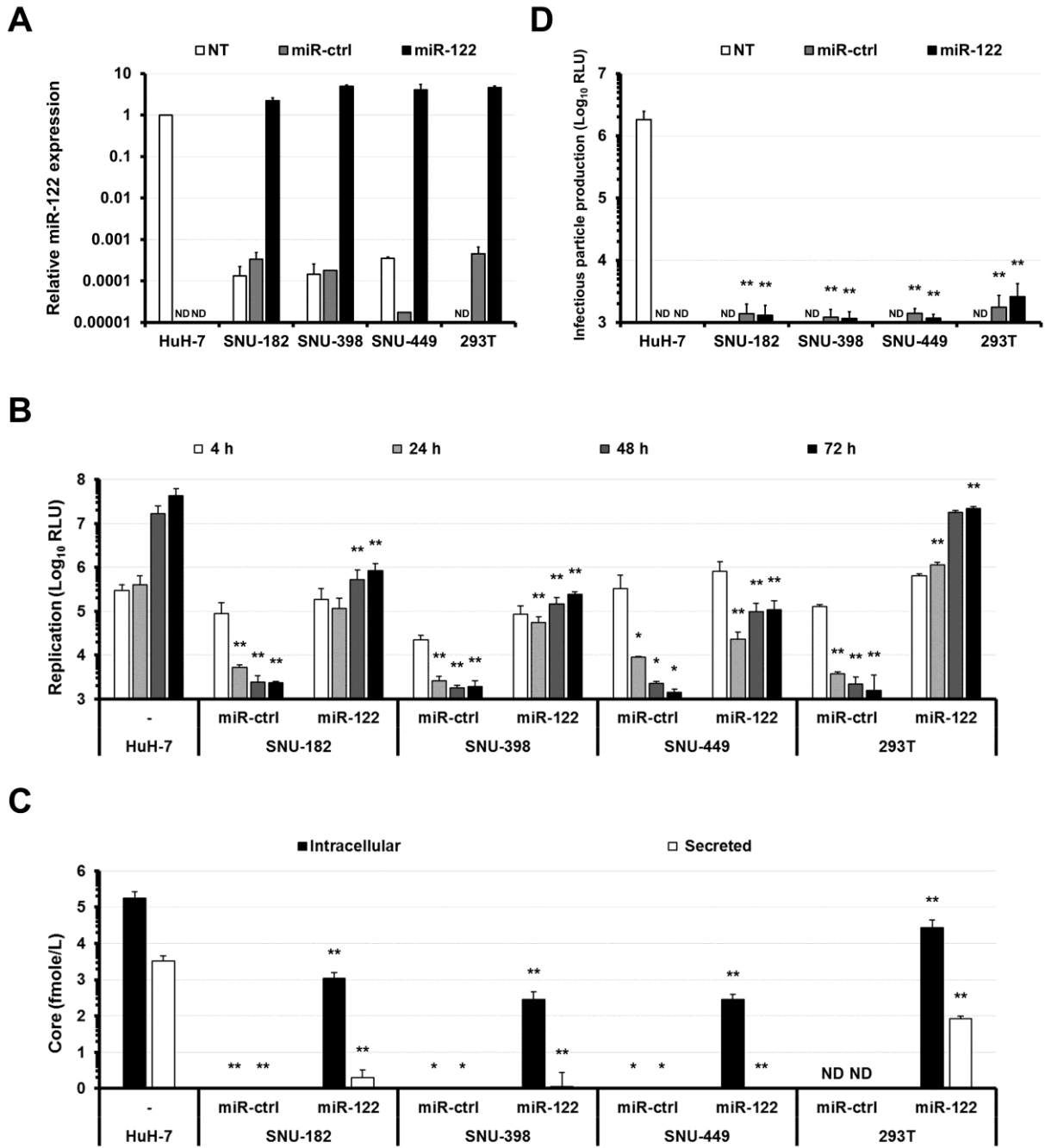


FIG 2

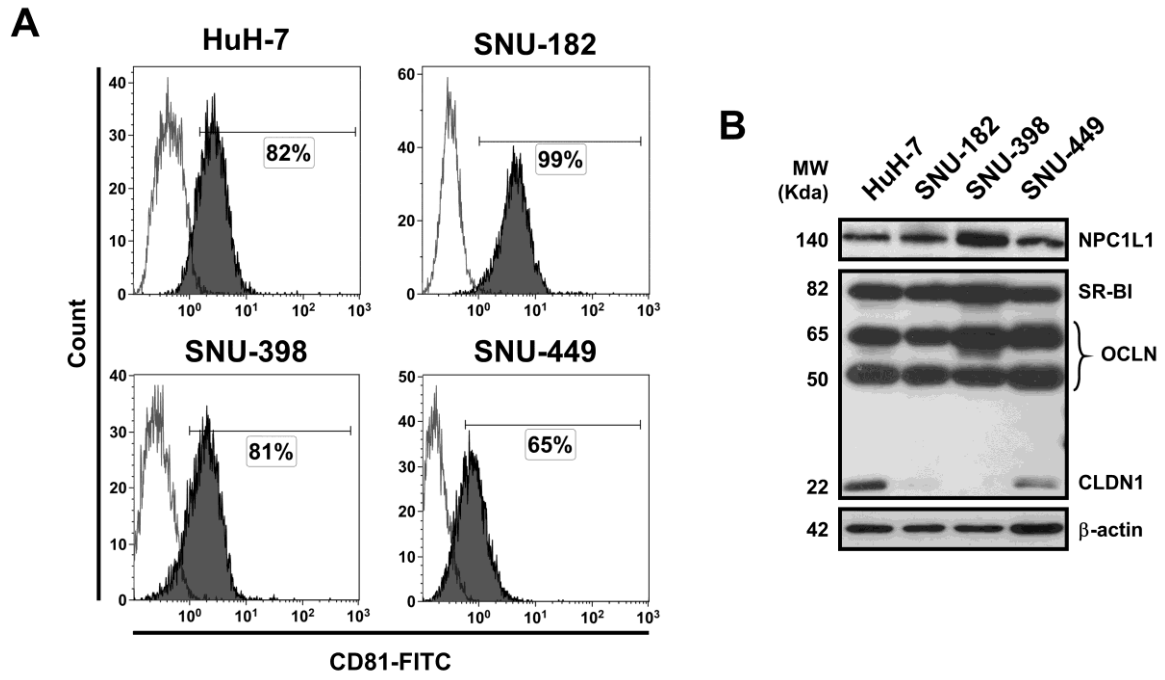


FIG 3

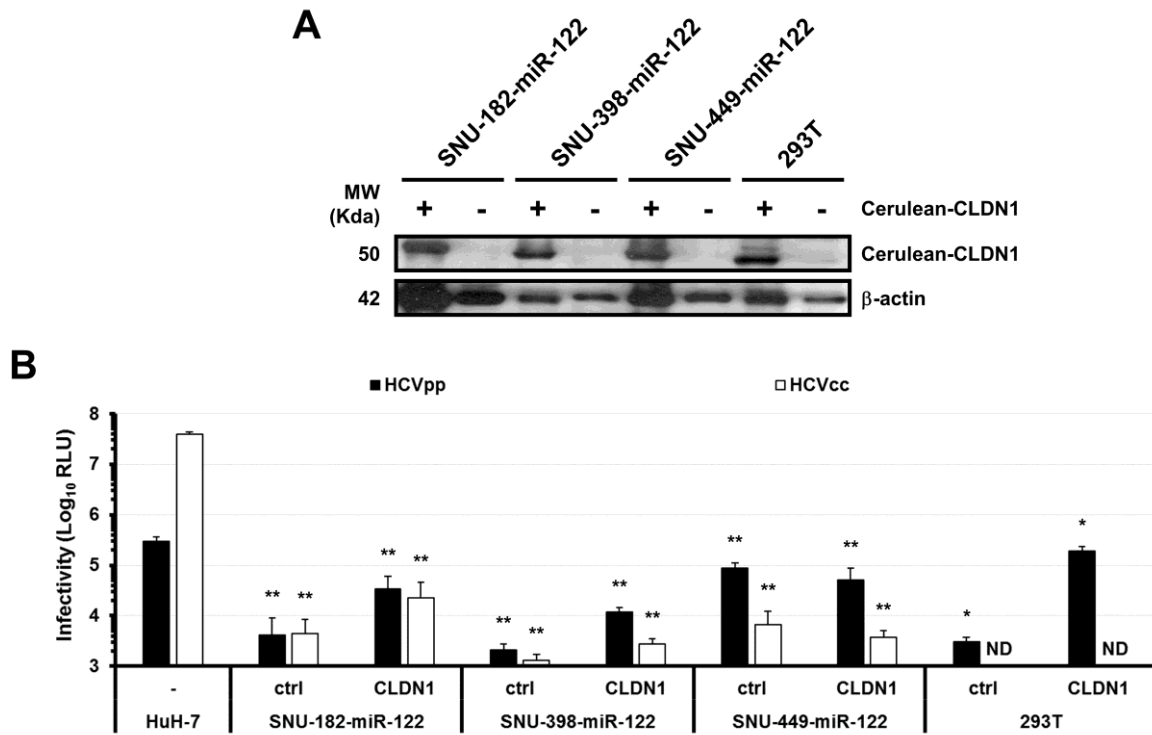


FIG 4

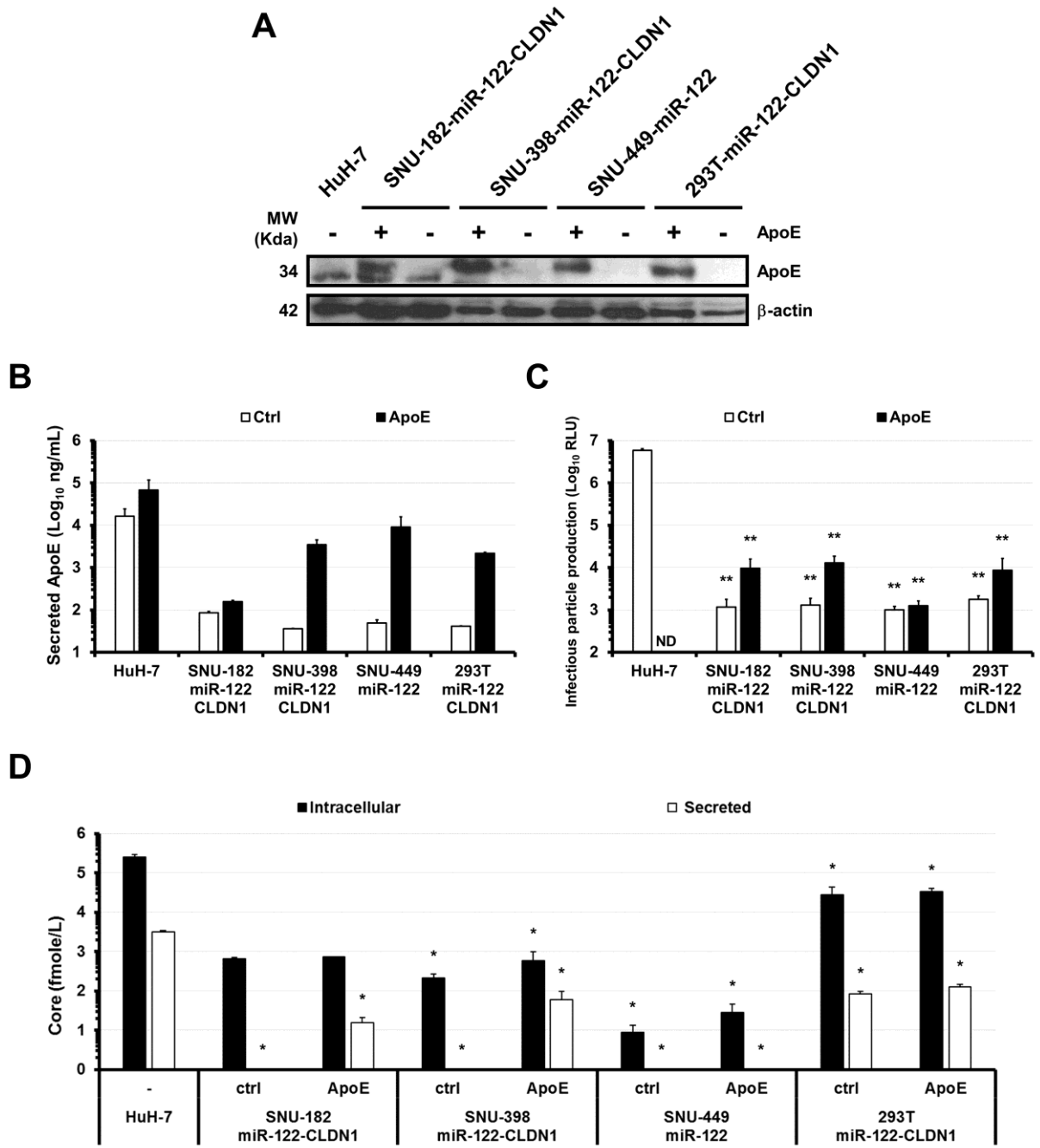


FIG 5