

## Reconstitution of the entire hepatitis C virus life cycle in non-hepatic cells.

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1 Short-Form Paper

2 **Reconstitution of the entire hepatitis C virus life cycle in non-hepatic cells**

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17

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22 **Author contribution:** MBZ and TFB designed and supervised research. DDC, MT, DJF,  
23 EG, SP, GL, RB, MBZ and TFB performed research. DDC, MT, EG, SP, MBZ and TFB  
24 analyzed data. RB provided important ideas for the initiation and execution of this study  
25 and provided reagents. DDC, MT, MBZ, DJF and TFB wrote the manuscript. DDC, MT,  
26 and DJF contributed equally to this work. MBZ and TFB contributed equally.

27

28 **Abbreviations:**

29 HCV, hepatitis C virus; HCVpp, retroviral HCV pseudo-particle; HCVcc, cell culture-  
30 derived HCV; OCLN, occludin; CLDN1, claudin-1; SR-BI, scavenger receptor class B  
31 type I; miR-122, microRNA-122; apoE, apolipoprotein E; EGFR, epidermal growth factor  
32 receptor; VLDL, very-low-density lipoprotein.

33

34 **Abstract**

35 Hepatitis C virus (HCV) is a human hepatotropic virus, yet the relevant host factors  
36 restricting HCV infection to hepatocytes are only partially understood. We demonstrate  
37 that exogenous expression of defined host factors reconstituted the entire HCV life cycle  
38 in human non-hepatic 293T cells. This study shows robust HCV entry, RNA replication,  
39 and production of infectious virus in human non-hepatic cells, and highlights key host  
40 factors required for liver tropism of HCV.

41

42 **Main Text**

43 Virus-host interactions that determine and restrict specific tissue and host tropisms  
44 display complex evolutionary history and have significant consequences on the  
45 pathogenesis of viral infection and human disease. Viral hepatitis is a major disease  
46 burden. Indeed, infection of hepatocytes by a variety of hepatotropic viruses from  
47 different orders and families can lead to tissue inflammation, fibrosis, and hepatocellular  
48 carcinoma. Hepatitis C virus (HCV), a member of the family *Flaviviridae*, is a prime  
49 example of a virus that causes chronic hepatitis worldwide. While HCV primarily infects  
50 hepatocytes of humans and chimpanzees, the virus has been shown to enter neuronal  
51 and endothelial cells of the blood-brain barrier. However, infection of these cells occurs  
52 at a low level and production of infectious viruses is greatly diminished relative to  
53 hepatically derived cells (9, 10). Unlike HCV, other members of the family *Flaviviridae*  
54 have a much broader tissue and species tropism. For example Dengue virus infects and  
55 replicates both in the midgut epithelia of *Aedes aegypti* mosquitoes, and in human  
56 monocytes and hepatocytes (20, 25, 39). Moreover, a virus closely related to HCV was  
57 recently identified from dogs' respiratory samples (18). A large panel of host factors  
58 required for HCV has been identified so far (36). However, the key host factors  
59 mediating liver tropism of the virus and allowing reconstitution of the viral life cycle in  
60 human cells is still only partially understood.

61 Taking advantage of our current knowledge of host factors involved in HCV  
62 infection, we sought to engineer a human kidney cell line (293T) to be capable of  
63 sustaining the entire HCV life cycle. The aim was to define those host factors that are

64 necessary and sufficient for allowing the HCV life cycle, in order to understand the liver  
65 tissue-specificity of HCV.

66 293T cells were obtained from ATCC and their identity was verified by genomic  
67 profile comparison to the LGC Standards database by short tandem repeat profiling as  
68 described (1) (Fig. 1A). In order to render them infectable by HCV, we used lentiviral  
69 vectors to express the four principal HCV host entry factors: claudin-1 (CLDN1), CD81,  
70 occludin (OCLN), and scavenger receptor class B type I (SR-BI) (2, 7, 34, 35) by using  
71 previously described expression constructs and methods (3, 24). Four 293T stable cell  
72 lines were selected to express either CLDN1 alone, CD81/OCLN with or without CLDN1,  
73 or CLDN1/CD81/OCLN together with SR-BI (293T-4R). After verifying stable expression  
74 of these proteins using receptor-specific antibodies (Fig. 1B), we infected these cells  
75 with HCV pseudoparticles expressing the envelope glycoproteins of HCV genotype 1b  
76 (HCVpp; HCV-J strain, described in (31)). While CLDN1 expression alone conferred  
77 limited permissiveness for HCV infection as previously described (7), expression of all  
78 four factors enhances HCV entry to a level that was around four-fold higher than  
79 Huh7.5.1 cells, which is the liver-derived model hepatoma cell line for studying HCV  
80 infection (Fig. 1C).

81 Genuine cell culture infection of HCV (HCVcc) was then investigated in 293T-4R  
82 cells using a chimeric virus composed of two genotype 2a isolates (designated Jc1 (19,  
83 32)) and engineered for Renilla luciferase expression (JcR2a; (38)). However, as shown  
84 in Fig. 2A, overcoming the HCV entry block was not sufficient for robust viral RNA  
85 replication in 293T cells.

86           Several studies have shown that microRNA (miR)-122 is a liver-specific host  
87 factor critical for HCV replication (5, 16, 17, 28). Since Northern blot analyses  
88 demonstrated non-detectable miR-122 expression in 293T-4R cells (Fig. 2C), we  
89 investigated whether exogenous miR-122 expression reconstituted viral RNA replication.  
90 Indeed, stable expression of this factor, by using miR-122 encoding lentiviruses in the  
91 293T-4R line, conferred the cells permissive for bona fide HCVcc infection, with  
92 replication to comparable levels as Huh7.5.1 cells as assessed by luciferase reporter  
93 activity (Fig. 2B). Further confirmation of genuine infection was garnered by observing  
94 similar infectivity (TCID<sub>50</sub>) with HCVcc (Jc1) without a reporter gene, by detecting  
95 expression of viral protein NS5A (Fig. 2B). We verified expression of miR-122 in  
96 transduced 293T-4R/miR122 cells, and the level was comparable to that of Huh7.5.1  
97 cells as assessed by Northern blot (Fig. 2C), and the cell proliferation rate of the  
98 different cell lines was similar (data not shown). Kinetics of HCV replication in 293T-  
99 4R/miR122 cells matched those of Huh7.5.1 cells, suggesting that aside from miR-122,  
100 cell factors present in human liver- and kidney-derived cells are equally efficient for  
101 replication as assayed by luciferase reporter gene expression (Fig. 2D). Expression of  
102 viral proteins in infected cells was further confirmed using HCV core-specific  
103 immunofluorescence (Fig. 2E) and flow cytometry (data not shown).

104           To further confirm whether viral entry and replication in stably transduced 293T  
105 cells is mediated by the same host and virus factors as in human Huh7.5.1 hepatoma  
106 cells, we used well-characterized entry and replication inhibitors. Antibodies directed  
107 against the HCV entry factors CD81, CLDN1, and SR-BI (JS-81, BD Biosciences, (11),  
108 Zahid et al., unpublished, respectively) were effective in inhibiting infection (Fig. 2F).

109 Moreover, both a polyclonal serum recognizing apolipoprotein E (apoE) (29), and a  
110 monoclonal antibody recognizing the LDL receptor binding domain of apoE (37)  
111 effectively neutralized HCV infection of 293T-4R/miR122 cells (Fig. 2F). The same was  
112 true for the recently identified HCV entry inhibitor, erlotinib, which targets the kinase  
113 activity of the host entry regulatory protein, epidermal growth factor receptor (EGFR)  
114 (Fig. 2F) (24). Likewise, well characterized inhibitors of HCV NS3 protease or  
115 polymerase, telaprevir (VX950) and mericitabine (R7128), impaired HCV replication in  
116 293T-4R/miR122 cells (Fig. 2F). These data demonstrate that HCVcc RNA replication in  
117 kidney-derived 293T-4R/miR122 cells is efficient, and dependent on similar mechanisms  
118 as in liver-derived Huh7.5.1 cells.

119         Despite efficient entry and RNA replication of 293T-4R/miR122 cells infected with  
120 recombinant HCVcc, these cells did not release infectious virions, suggesting that  
121 kidney-derived cells lack factors required for viral assembly and release. Therefore, we  
122 aimed to reconstitute virus production by expression of HCV assembly factors. HCV  
123 production shares factors involved in very-low-density lipoprotein (VLDL) assembly, a  
124 process that occurs exclusively in hepatocytes (13, 14, 27). While the necessity of  
125 apolipoprotein B (apoB) in HCV production is controversial (15), apoE is known to be  
126 critical, and is incorporated into the virion (26). We therefore expressed the most  
127 common isoform of apoE (apoE3) in 293T-4R/miR122 cells by using a lentiviral vector  
128 encoding human apoE3 as described previously (23), and confirmed its expression by  
129 flow cytometry using an apoE-specific antibody (Fig. 3A). We then infected 293T-  
130 4R/miR122/apoE cells. Subsequently, the production and release of viral particles was  
131 assessed by incubating naïve Huh7.5.1 cells with the supernatants from these cells.



132 Indeed, 293T-4R/miR122/apoE released infectious HCV particles as shown by a marked  
133 and highly significant increase in infectivity (as assessed by luciferase activity of JcR2a  
134 virus and TCID<sub>50</sub> of Jc1 virus without a reporter gene) of the supernatant compared to  
135 the supernatant of 293T-4R/miR122 cells without apoE expression (Fig. 3B). Although  
136 the production of infectious particles was lower than in Huh7.5.1 cells studied in side-by-  
137 side experiments, these data indicate that apoE is a key factor for virus production in  
138 reconstituting the viral life cycle in non-hepatic cells. This diminished HCV production  
139 was not due to diminished replication levels as apoE transduced cells had similar HCV  
140 replication levels to 293T-4R/miR122 cells prior to apoE expression (data not shown).  
141 To test if HCV produced by these cells is reliant only on human apoE3 isoform or could  
142 use other forms of apoE, we similarly transduced human apoE2 and apoE4 isoforms, as  
143 well as murine apoE (Fig. 3C). Viruses produced from 293T cells expressing these apoE  
144 isoforms and the mouse ortholog had similar infectivity compared to human apoE3  
145 isoform (Fig. 3D).

146 Focusing on the most common apoE isoform (apoE3), we further characterized  
147 the kinetics and attributes of these viruses. First, we confirmed that HCV particles from  
148 engineered 293T cells could establish infection by monitoring the increase in HCV  
149 genomes over time in Huh7.5.1 target cells after exposure to the supernatant of HCVcc-  
150 infected 293T-4R/miR122/apoE cells (Fig. 4A). Next, we characterized the kinetics of  
151 HCV RNA production from infected 293T-4R/miR122/apoE cells by measuring HCV  
152 RNA in the media at serial time points following infection (Fig. 4B). Interestingly, the  
153 levels of HCV RNA released into the culture media of 293T-4R/miR122/apoE cells was  
154 similar to levels of HCV RNA in the media of Huh7.5.1 cells after 72h, whereas cells that

155 were not transduced with apoE released minimal amounts of HCV RNA, likely due to  
156 previously reported non-specific release of HCV RNA during replication (Fig. 4B)(33).  
157 These data suggest that the specific infectivity differs between virus produced from  
158 Huh7.5.1 cells and 293T cells engineered to express essential host factors. An  
159 estimation of the specific infectivity of the released viruses (TCID<sub>50</sub>/HCV RNA genomes)  
160 revealed approximately a 30-fold difference between the differently derived viruses  
161 (1/900 for Huh7.5.1-derived virus and 1/26,000 for 293T-4R/miR122/apoE-derived  
162 virus). It should be noted that HCV particles produced from 293T-4R/miR122/apoE cells  
163 proved to have a similar route of infection to hepatically-derived HCVcc, in that entry into  
164 Huh7.5.1 cells was neutralized by well-characterized HCV entry inhibitors including  
165 CD81-, SR-BI-, CLDN1-, apoE-specific antibodies, and erlotinib (Fig. 4C). Fractionating  
166 the virus by iodixanol density gradients revealed that the infectious virions produced  
167 from 293T-4R/miR122/apoE cells have similar buoyant density as those from Huh7.5.1  
168 cells (Fig. 4D).

169 The data presented here demonstrate that trans-expression of OCLN, CD81,  
170 CLDN1, SR-BI, miR-122, and apoE endow 293T human kidney-derived cells with the  
171 capacity to support the complete HCV life cycle. Expression of four principal entry  
172 factors and miR-122 generated cells with higher entry and similar replication kinetics as  
173 the extensively optimized Huh7.5.1 cells (4, 41). It should be noted in this context, that  
174 the recently identified entry factor EGFR is also expressed in 293T cells (data not  
175 shown, 24, 40). We confirmed that expression of CLDN1 alone appears to be sufficient  
176 for infection of 293T cells (7), and expand these findings in that high-level expression of  
177 the four canonical HCV entry factors make previously impenetrable cells four-fold more

178 permissive than Huh7.5.1 cells. These observations were confirmed by HCVcc infection  
179 of 293T cells engineered to express miR-122 in addition to variable sets of entry factors  
180 (data not shown). While the present study focused on engineering a human cell line for  
181 infection, it has been demonstrated that concomitant high level expression of the four  
182 human entry factors is required for robust entry of mouse hepatocytes *in vivo* (6). Since  
183 none of the identified entry factors are exclusively expressed in the liver, it is likely that  
184 the combined expression of these host factors at substantial levels allows the virus to  
185 productively infect the human liver, rather than a single liver-specific entry factor  
186 restricting HCV infection.

187         Investigators have shown that miR-122 expression increases HCV replication in  
188 mouse embryonic fibroblasts and other hepatoma cell lines such as HepG2 cells (21, 17,  
189 28). Furthermore, HEK-293 cells modified to express miR-122 are capable of sustaining  
190 selectable HCV subgenomic replicons, although expression of mutated miR-122, at sites  
191 required for HCV RNA binding, can also sustain these replicons (5). We demonstrate  
192 here *de novo* replication following an infection event of a non-hepatic cell line  
193 engineered to express HCV host factors. Our data also demonstrate that there is no  
194 restrictive factor of HCV entry and viral RNA replication that is present in 293T cells.  
195 HCV entry and replication in human blood brain barrier endothelial and neuronal cells  
196 have been described (9, 10). In contrast to the kidney-derived cells described here, HCV  
197 replication in blood brain barrier endothelial cells occurred via a miR-122 independent  
198 mechanism, yet at a diminished level (9). Thus, the cell lines developed in this study  
199 may be useful as a tool to further understand the molecular mechanisms of extra-hepatic  
200 infection.

201 The production of HCV from 293T-4R/miR122/apoE cells was diminished relative  
202 to Huh7.5.1 cells, but markedly and significantly higher than in cells without apoE  
203 expression. This demonstrates that apart from apoE, all the other factors necessary for  
204 the production of infectious particles are present in 293T cells, yet additional host factors  
205 may increase efficient production levels. The cell line generated in this study is likely to  
206 allow further discovery of the minimal set of host factors required for robust viral  
207 production. Additional relevant factors enhancing viral production may be apoB (27),  
208 DGAT1 (13), or microsomal triglyceride transfer protein (MTP) (12, 14). Notably, apoE  
209 has recently been demonstrated to be essential for virus production; apoE-deficient  
210 mouse hepatocytes with trans-expression of HCV RNA and proteins along with apoE are  
211 able to produce high levels of infectious virions (23).

212 In summary, this study demonstrates that a small set of defined host factors is  
213 sufficient to reconstitute the complete viral life cycle in non-hepatic cells. These results  
214 advance our knowledge on tissue-specific factors for HCV infection and provide novel  
215 tools to elucidate host and restriction factors for the HCV life cycle.

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230

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- 373

374 **Figure Legends**

375 **Figure 1. Expression of four HCV entry factors renders 293T cells highly**  
376 **permissive to HCVpp entry.** (A) Short tandem repeat (STR) profile of the 293T cells  
377 used in this study (Cell line authentication, LGC Standards) was performed as described  
378 previously (1). The names of tested loci are indicated in bold and peak positions from  
379 STR profile of 293T cells were compared to LGC Standards database. (B) 293T cells  
380 (cultured in DMEM high glucose, Life Tech) were transduced with lentiviruses (as  
381 described in (3)) to express given HCV entry factors. After transduction, cells were  
382 selected with blasticidin (12 µg/ml) for 2 weeks. Blasticidin-resistant cells were assessed  
383 by flow cytometry using monoclonal antibodies (CLDN1 (11), OCLN (Cat.# 33-1500  
384 Invitrogen), SR-BI (Zahid et al., submitted manuscript)) recognizing indicated entry  
385 factors. Entry factor transduced cells (dark grey histograms) were compared to naïve  
386 293T cells (light grey histograms) and isotype control antibody (Cat.# 10400C, Life  
387 Technologies, white dashed histograms). X axis: fluorescence intensity, Y axis: number  
388 of events. (C) Transduced 293T cells were assessed for HCVpp (genotype 1b; HCV-J  
389 strain; produced as described in (31)) entry by determining luciferase activity 72h post-  
390 infection as previously described (35). Results were first normalized to vesicular  
391 stomatitis virus pseudoparticle entry (VSV-Gpp; produced as described in (8)), and then  
392 compared to Huh7.5.1 cells (cultured as described in (41)). Results are expressed as  
393 means +/- SD of percentage HCVpp entry relative to entry into Huh7.5.1 cells from three  
394 independent experiments performed in triplicate, and 100% relative infectivity is  
395 represented by a solid line. Statistical analysis for entry factor expressing cells relative to  
396 naïve 293T cells was performed using the Student's *t* test, \*P<0.05.

397

398 **Figure 2. 293T-4R cells support robust HCV infection upon miR-122 expression.**

399 (A) Stable 293T-4R cells described in Fig. 1 were challenged with HCVcc (JcR2a;  
400 produced as described in (38)) or were mock infected and luciferase activity was  
401 assessed 72h post-infection as described previously (38). Results are expressed as  
402 means +/- SD of relative light units (RLU) from three independent experiments  
403 performed in triplicate. (B) 293T-4R cells were stably transduced using miR-122  
404 encoding lentiviruses (Cat.# mh15049, ABM Good) and puromycin (2,5 µg/ml) resistant  
405 cells were selected over 2 weeks. 293T-4R/miR122 cells and Huh7.5.1 cells were then  
406 infected with HCVcc or mock infected for 6h. Infection was assayed by monitoring  
407 luciferase activity 72h post-infection. Results are expressed as means +/- SD of relative  
408 light units (RLU) from three independent experiments performed in triplicate. Jc1, an  
409 HCVcc without a luciferase reporter (32) was likewise used to infect Huh7.5.1 and 293T-  
410 4R/miR122 cells and its infectivity was assessed by limiting-dilution assay (TCID<sub>50</sub>) by  
411 detecting viral protein NS5A using immunohistochemistry, represented as grey bars  
412 (22). Results are expressed as means +/- SD of TCID<sub>50</sub>/ml from three independent  
413 experiments. (C) Northern blots of miR-122 and miR-16, and U6 RNA as a loading  
414 control, extracted from 293T-4R, 293T-4R stably expressing miR-122, and Huh7.5.1  
415 cells as positive control. Northern blots using a miR-122-specific probe were performed  
416 as described previously (30). Oligonucleotide lengths (nt) are indicated on the left of  
417 each blot. (D) 293T-4R, 293T-4R/miR122 and Huh7.5.1 cells were incubated side-by-  
418 side with HCVcc (JcR2a) and luciferase activity was monitored every 24h over a 72 h  
419 period. Results are expressed as means +/- SD of relative light units (RLU) of three  
420 independent experiments performed in triplicate. (E) Huh7.5.1, 293T-4R, and 293T-

421 4R/miR122 cells were infected for 72 h and HCV core protein (core antibody C7-50,  
422 Thermo Scientific,), or non-specific IgG, as a control (Cat.# 10400C, Life Technologies)  
423 were observed by immunofluorescence; nuclei were stained using DAPI. (F) 293T-  
424 4R/miR122 cells were pre-incubated for 1h at 37°C with the indicated entry inhibitors,  
425 antivirals or controls (monoclonal antibodies (mAb), anti-CD81 (JS81, BD Biosciences),  
426 anti-CLDN1 (11), anti-SR-BI (Zahid et al. submitted manuscript), polyclonal (pAb) anti-  
427 apoE (Cat #178479, Calbiochem), anti-apoE mAb was described in (37), 20 µg/ml,  
428 erlotinib: 10 µM (Cat.# E-4997, LC Laboratories), protease inhibitor telaprevir VX950: 1  
429 µM; polymerase inhibitor mericitabine R7128: 1 µM; both synthesized by Acme  
430 Bioscience Inc. , DMSO: 0.7%, and then infected with HCVcc (JcR2a) in the presence of  
431 given entry inhibitors or antivirals. Cell lysates were assessed for luciferase activity 72h  
432 post-infection. Results are expressed as means +/- SEM of percentage HCVcc infection  
433 compared to controls, from three independent experiments performed in triplicate, and  
434 100% relative infectivity is represented by a solid line. In panels A, B, and D, detection  
435 limits are represented by dashed lines. Statistical analysis relative to control was  
436 performed using the Student's *t* test, \*P<0.05.

437  
438 **Figure 3. Infectious HCV particles are released from 293T-4R/miR122 cells upon**  
439 **apoE expression.** (A) 293T-4R/miR122 cells were transduced with an apoE3 encoding  
440 lentiviral vector described in (23). 72h post-transduction, cells that were or were not  
441 transduced were stained for flow cytometry analysis. ApoE expression was analyzed  
442 using a specific apoE antibody (clone D6E10, Cat.# ab1906, Abcam, untransduced cells  
443 are represented as light grey histogram and transduced cells are shown as dark grey  
444 histogram) and an isotype antibody (Cat.# 10400C, Life Technologies) was used as

445 control (white dashed histograms). Huh7.5.1 cells were used for control of apoE  
446 expression and PBS is presented as control of the isotype antibody (thick black  
447 histogram). (B) Transduced 293T-4R/miR122/apoE cells were infected with HCVcc  
448 (JcR2a, or Jc1). 6h post-infection, cells were washed three times with PBS, and fresh  
449 culture medium was added. 72h post-infection, media from infected cells was passaged  
450 onto naïve Huh7.5.1 cells. Cell lysates of JcR2a infected cells were assessed for  
451 luciferase activity 72h post-infection. Results are expressed as means +/- SD of relative  
452 light units (RLU) of three independent experiments performed in triplicate. The detection  
453 limit is represented by a dashed line. The infectivities of Jc1 derived from Huh7.5.1 or  
454 293T-4R/miR122/apoE infected cells were assessed by limiting-dilution assay (TCID<sub>50</sub>)  
455 by detecting NS5A by immunohistochemistry, represented as grey bars. Results are  
456 expressed as means +/- SD of TCID<sub>50</sub>/ml from three independent experiments. #  
457 represents below detectable levels. Statistical analysis relative to the control was  
458 performed using the Student's *t* test, \*P<0.05. (C) 293T-4R/miR122 cells were  
459 transduced with indicated apoE isoform-encoding lentiviral vectors (24), or mock  
460 transduced (Control). 72h post-transduction, cells were either lysed or seeded for  
461 HCVcc infection. Cell lysates were assessed for apoE expression by Western blot either  
462 by using apoE antibody (clone D6E10, Cat.# ab1906, Abcam) for human apoE (h-apoE)  
463 expression or using a mouse apoE specific antibody for mouse apoE (m-apoE)  
464 expression (Cat# ab20874, Abcam). Huh7.5.1 and primary mouse hepatocytes (PMH)  
465 were used as controls for human and mouse apoE expression, respectively. (D) The  
466 different apoE isoform-expressing 293T-derived cells were assessed for their capacity to  
467 produce infectious virus by infecting them with HCVcc (JcR2a) and 72h post-infection,  
468 supernatants of infected 293T-derived cells were passaged onto naïve Huh7.5.1 cells.



469 72h after initiating this infection, Huh7.5.1 cells were lysed and luciferase activity  
470 assessed. Results are expressed as means +/- SD of relative light units (RLU) from a  
471 representative experiment performed in triplicate. The dashed line represents the  
472 detection limit.

473  
474 **Figure 4. Characterization of HCVcc derived from 293T-4R/miR122/apoE cells (A)**

475 Culture media from Jc1-infected 293T-4R/miR122, 293T-4R/miR122/apoE, and  
476 Huh7.5.1 cells were passaged onto naïve Huh7.5.1 target cells. Total RNA from these  
477 Huh7.5.1 target cells was extracted at indicated time points and HCV RNA was  
478 quantitated by RT-qPCR as described (11). Values were normalized to the internal  
479 control gene GAPDH and are represented as HCV RNA to GAPDH RNA ratio. Results  
480 are expressed as means +/- SD from an experiment performed in quadruplicate. (B)  
481 HCV RNA production was measured by infecting 293T-4R/miR122, 293T-  
482 4R/miR122/apoE and Huh7.5.1 cells side-by-side with HCVcc (Jc1). RNA from  
483 supernatants of infected cells was extracted at indicated time points and HCV RNA  
484 quantitated by RT-qPCR. Results are expressed as means +/- SD of copies/ml from an  
485 experiment performed in triplicate. (C) Culture media of infected 293T-4R/miR122/apoE  
486 cells were harvested 72h post-infection and passaged onto naïve Huh7.5.1 cells that  
487 were pre-incubated with either control IgG, DMSO, or with indicated entry inhibitors.  
488 Results represent mean percentages of HCV infection (as assessed by luciferase  
489 activity) relative to control +/- SD from a representative of two independent experiments  
490 performed in triplicate, and 100% relative infectivity is represented by a solid line. Virus  
491 used was JcR2a with a TCID<sub>50</sub> of 10<sup>5</sup> to 10<sup>6</sup>/ml. (D) Density distributions of infectious  
492 293T-4R/miR122/apoE- and Huh7.5.1-derived HCVcc (Jc1) were determined by

493 overlaying 0.5 ml of culture media on a 5 ml, 4-40% iodixanol step gradient, and  
494 ultracentrifuging samples for 16h at 40,000 rpm on a SW-55 rotor (Beckman Coulter).  
495 Fractions were carefully harvested from the top of each tube, and density was  
496 determined by weighing 0.5 ml of each fraction. Each fraction was assayed for infectivity  
497 by TCID<sub>50</sub> by detecting NS5A as described (22).

498

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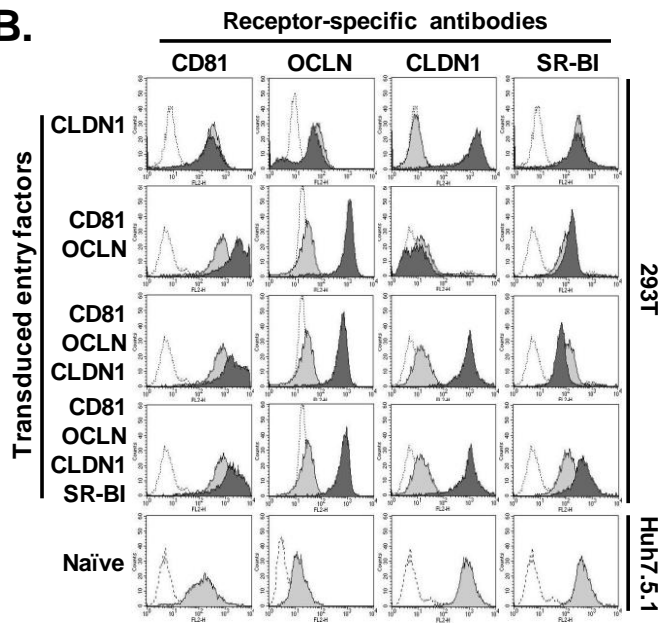
**A.**

Loci Tested	AMELO	THO1	D5	D13	D7
ATCC Reference: CRL-1573 (HEK293)	X, X	7, 9.3	8, 9	12, 14	11, 12
293T cells	X, X	9.3, 9.3	8, 9	12, 13, 14	11, 12

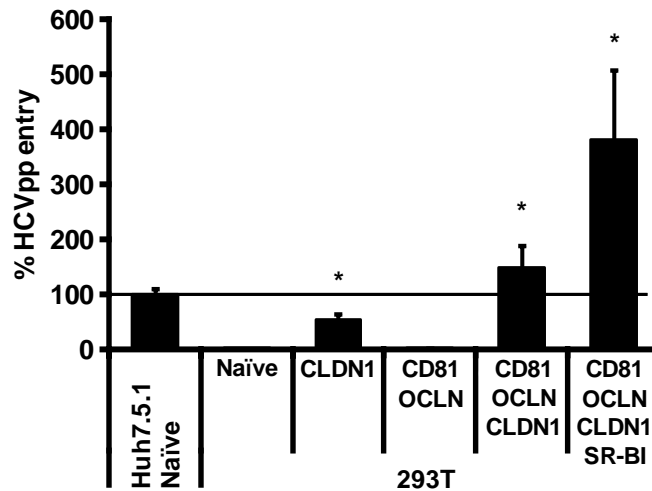
  

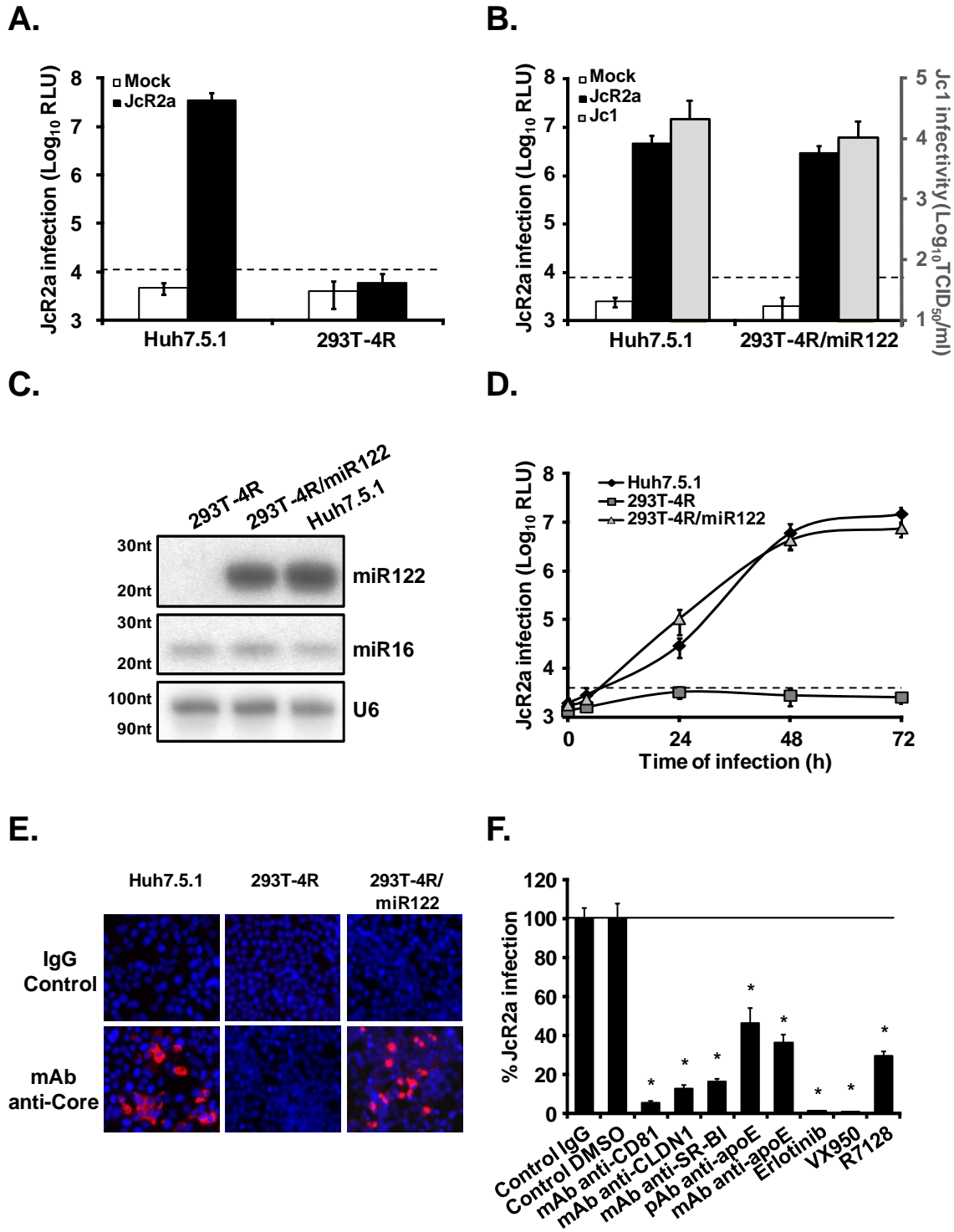
Loci Tested	D16	CSF	VWA	TPOX
ATCC Reference: CRL-1573 (HEK293)	9, 13	11, 12	16, 19	11, 11
293T cells	9, 13	11, 12	16, 19	11, 11

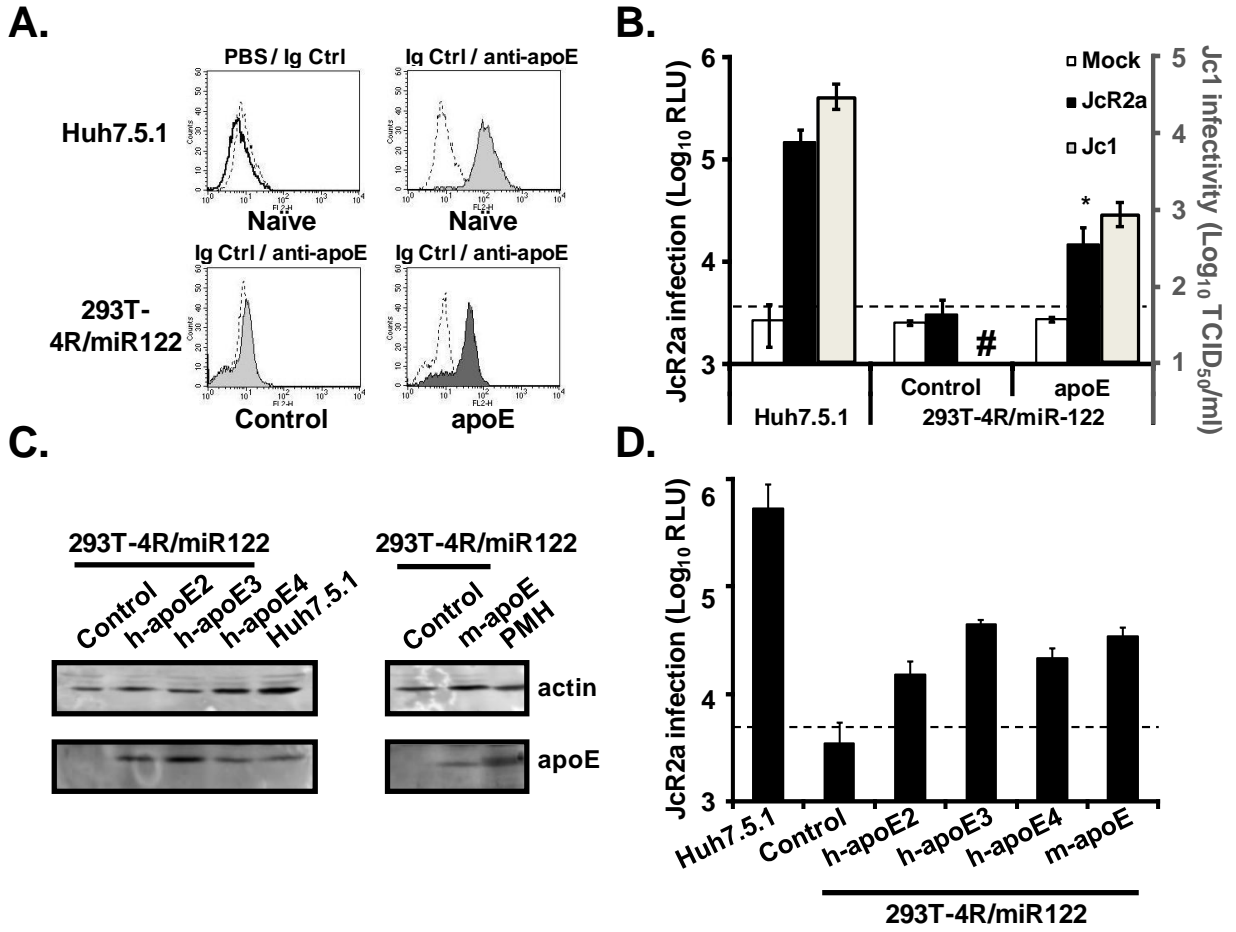
**B.**



**C.**







505

506

