



**HAL**  
open science

## Loss of hepatitis D virus infectivity upon farnesyl transferase inhibitor treatment associates with increasing RNA editing rates revealed by a new RT-ddPCR method

Eloi R Verrier, Anna Salvetti, Caroline Pons, Maud Michelet, Michel Rivoire, Thomas F Baumert, David Durantel, Julie Lucifora

### ► To cite this version:

Eloi R Verrier, Anna Salvetti, Caroline Pons, Maud Michelet, Michel Rivoire, et al.. Loss of hepatitis D virus infectivity upon farnesyl transferase inhibitor treatment associates with increasing RNA editing rates revealed by a new RT-ddPCR method. *Antiviral Research*, 2022, 198, pp.105250. 10.1016/j.antiviral.2022.105250 . hal-03540952

**HAL Id: hal-03540952**

**<https://hal.science/hal-03540952v1>**

Submitted on 24 Jan 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# 1 **Loss of hepatitis D virus infectivity upon Farnesyl transferase inhibitor** 2 **treatment associates with increasing RNA editing rates revealed by a new** 3 **RT-ddPCR method**

4  
5 Eloi R. Verrier<sup>1,\*</sup>, Anna Salvetti<sup>2</sup>, Caroline Pons<sup>2</sup>, Maud Michelet<sup>3</sup>, Michel Rivoire<sup>4</sup>, Thomas F.  
6 Baumert<sup>1,5</sup>, David Durantel<sup>2</sup> and Julie Lucifora<sup>2,\*</sup>

7  
8 <sup>1</sup> Université de Strasbourg, Inserm, Institut de Recherche sur les Maladies Virales et Hépatiques  
9 UMR\_S1110, Strasbourg, France.

10 <sup>2</sup> CIRI - Centre International de Recherche en Infectiologie, Univ Lyon, Université Claude Bernard Lyon  
11 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon, F-69007 Lyon, France.

12 <sup>3</sup> Inserm, U1052, Cancer Research Center of Lyon (CRCL), Université de Lyon (UCBL1), CNRS  
13 UMR5286, Centre Léon Bérard, Lyon, France.

14 <sup>4</sup> INSERM, U1032, Centre Léon Bérard (CLB), Lyon, France

15 <sup>5</sup> Institut Hospitalo-Universitaire, Pôle Hépatogastro-digestif, Nouvel Hôpital Civil, Strasbourg, France.

16  
17 \* corresponding authors: [e.verrier@unistra.fr](mailto:e.verrier@unistra.fr); [julie.lucifora@inserm.fr](mailto:julie.lucifora@inserm.fr)  
18  
19  
20

## 21 **Abstract**

22 Chronic hepatitis D is the most severe form of chronic viral hepatitis and to date, efficient therapeutic  
23 approaches against hepatitis D virus (HDV) are absent. Among the antiviral molecules currently tested  
24 in clinical trials, the farnesyl transferase inhibitor (FTI) Lonafarnib inhibits the prenylation of the large  
25 delta antigen (L-HDAg), blocking virus assembly. Given the importance of L-HDAg in the virus life cycle,  
26 we hypothesized that Lonafarnib treatment may have side effects on virus replication. Here, we setup  
27 an innovative method for the quantification of HDV RNA allowing the independent quantification of edited  
28 and non-edited versions of the HDV genome upon infection. We demonstrated that FTI treatment of  
29 HBV/HDV co-infected dHepaRG or primary human hepatocytes leads to an accumulation of intracellular  
30 HDV RNAs and a marked increase in the levels of edited RNAs non only within the infected cells but  
31 also in the viral particles that are produced. Interestingly, these viral particles were less infectious,  
32 probably due to an enrichment in edited genomes that are packaged, leading to unproductive infection  
33 given the absence of S-HDAg synthesis after viral entry. Taken together, we setup an innovative  
34 quantification method allowing the investigation of RNA editing during HDV infection in a simple, fast,  
35 clinically-relevant assay and demonstrated for the first time the dual antiviral activity of FTI on HDV  
36 infection.

## 37 38 **Keywords**

39 Hepatitis D virus, RT-ddPCR, antiviral treatment, FTI, hepatocytes

## 40 **Short Communication**

41 Chronic hepatitis D is the most severe form of chronic viral hepatitis (Koh et al., 2019). It is caused by  
42 the co-infection of hepatocytes with hepatitis B virus (HBV) and its satellite virus, hepatitis D virus (HDV)  
43 (Lucifora and Delphin, 2020). To date, no treatment allows viral cure in chronic HDV patients (Turon-  
44 Lagot et al., 2020). Moreover, their management remains challenging due to the absence of gold  
45 standard biomarker for either response to treatment or liver disease progression. HDV is a small virus  
46 characterized by a single-stranded circular RNA genome. The single open reading frame encodes the  
47 short and long forms of the delta antigen (S-HDAg, L-HDAg, respectively) (Lucifora and Delphin, 2020).  
48 S-HDAg is initially synthesized upon transcription of short mRNAs. ADAR-mediated edition of the amber  
49 stop codon in the open reading frame on the antigenome triggers the production of a longer mRNA  
50 leading to L-HDAg synthesis (Lucifora and Delphin, 2020). In addition to their structural function, both  
51 antigens play antagonistic roles during viral replication: while S-HDAg is essential for the replicative  
52 phase, L-HDAg prenylation leads to its inhibition and the initiation of virion assembly through the  
53 interaction with HBV envelop proteins (morphogenetic phase) (Glenn et al., 1992; Lucifora and Delphin,  
54 2020; Sato et al., 2004). As a consequence, it is suspected that virions containing edited genomes (that  
55 can be packaged into viral particles as much as non-edited genomes in the current state of our  
56 knowledge) would be non-infectious since replication cannot occur in absence of S-HDAg synthesis  
57 (Casey, 2012). Among the current anti-HDV antivirals that are evaluated in clinical trials, farnesyl  
58 transferase inhibitor (FTI) Lonafarnib inhibits virion assembly by preventing L-HDAg prenylation (Turon-  
59 Lagot *et al.*, 2020). Although its effect on viral assembly is well characterized, its interaction with the  
60 other steps of the virus life cycle is not totally understood. We and others previously reported an  
61 accumulation of intracellular HDV RNA upon FTI treatment, probably because prenylation is essential  
62 for L-HDAg-mediated inhibition of HDV replication (Alfaiate et al., 2016; Lempp et al., 2019; Sato *et al.*,  
63 2004). We hypothesize that this accumulation may lead to a change in the ratio between edited and  
64 non-edited genomes, that may have consequences on the reminiscent particles produced by FTI-treated  
65 cells. Here, we investigated the impact of FTI treatment on HDV replication using a novel method based  
66 on reverse transcription digital droplet PCR (RT-ddPCR) (Hindson et al., 2011).

67 We first designed a specific duplex assay using primers amplifying the editable region of the  
68 HDV genome (Amber codon) and two dual-labelled probes specific for edited and non-edited HDV  
69 sequences that differ from one single nucleotide (Supporting information). Duplex ddPCR analyses

70 using increasing amounts of non-edited or edited HDV RNA-derived DNA templates in separate assays  
71 (Figure 1A) or mixed templates containing variable proportions of edited and non-edited fragments  
72 (Figure 1B) demonstrated the ability of our assay to discriminate both forms of HDV genome regardless  
73 the initial ratio between edited and non-edited copies. To validate this assay in a biological context, we  
74 decreased the levels of *ADAR* expression through an RNA interference strategy in order to modify the  
75 ratio between non-edited and edited HDV RNAs. Huh7.5-NTCP cells were transfected with *ADAR*-  
76 specific siRNA before co-infection with HBV and HDV. As expected, *ADAR* knock-down (Figure 1C-1D)  
77 was associated with (i) a decrease in L-HDAg levels compared to cells transfected with control siRNA  
78 (Figure 1C) and (ii) an increase in the levels of HDV RNAs, the latter probably due to the reduction of L-  
79 HDAg-mediated inhibition of HDV replication (Figure 1E). The duplex RT-ddPCR analyses revealed an  
80 increase in non-edited HDV copy level in *ADAR*-silenced cells compared to control cells (Figure 1F)  
81 confirming the relevance of our assay in a biological context. Of note, when adding the numbers of  
82 edited and non-edited copies detected by RT-ddPCR, total HDV RNA production followed the same  
83 dynamics throughout the infection as the one detected by conventional RT-qPCR (Figure 1E and 1F).  
84 In addition, silencing of *ADAR* expression had no effect on HBV replication (Figure 1G).

85 We next applied this strategy to evaluate whether the inhibition of L-HDAg prenylation may affect  
86 the ratio between edited and non-edited HDV sequences that accumulate during viral replication. To do  
87 so, differentiated HepaRG cells (dHepaRG) or primary human hepatocytes (PHH) from two donors were  
88 co-infected with HBV and HDV and treated with FTI-277 (Figure 2). RT-qPCR analyses revealed an  
89 increase in HDV RNAs upon treatment as previously described (Alfaiate *et al.*, 2016) (Figure 2A).  
90 Interestingly, accumulation of HDV RNAs was associated to an increase in edited/non-edited ratio in  
91 FTI-treated cells (Figure 2B). In the supernatant of co-infected cells, while FTI-277 treatment induced  
92 an expected decrease in HDV particle production by inhibiting virus assembly (Figure 2C), an increased  
93 number of edited versions of the HDV genome was detected by RT-ddPCR (Figure 2D). To assess  
94 whether the modulation of this ratio would affect virus particle infectivity, adjusted viral genome  
95 equivalents (vge) harvested from the supernatant of treated and untreated co-infected dHepaRG cells  
96 or PHH were used to infect naive Huh7.5-NTCP cells. As shown in Figure 2E-F, a marked decrease in  
97 HDV infection was observed using particles from FTI-treated supernatants, suggesting a decrease in  
98 virus infectivity due to an accumulation of defective viral particles containing edited versions of the HDV  
99 genome.

100 Taken together, we described a novel RT-ddPCR method that allows the distinction between  
101 edited and non-edited versions of the HDV genome upon infection in a single and faster assay compared  
102 to existing methods based on RNA sequencing (Dziri et al., 2021). The validation of this assay using  
103 serum from infected patients would help to determine the clinical relevance of HDV editing rate for  
104 disease progression or response to treatment. Moreover, we confirmed that FTI treatment of infected  
105 cells induces an accumulation of HDV genomes likely due to a blockage of L-HDAg-mediated inhibition  
106 of viral replication, which depends on L-HDAg prenylation as it has been previously suggested (Sato *et*  
107 *al.*, 2004). Due to continuous ADAR activity, this accumulation would affect more and more edited  
108 versions of the genomes leading to an increase in the edited/non-edited ratio. Our study reveals for the  
109 first time a putative dual antiviral activity of FTI on HDV infection by decreasing not only the number but  
110 also the specific infectivity of viral particles produced by co-infected cells. Although our results would  
111 benefit from being validated for all HDV genotypes and in treated patients, this study paves the way  
112 toward a comprehensive and clinically-relevant understanding of Lonafarnib antiviral mode of action.

113

#### 114 **References**

115 Alfaiate, D., Lucifora, J., Abeywickrama-Samarakoon, N., Michelet, M., Testoni, B., Cortay, J.C., Sureau,  
116 C., Zoulim, F., Deny, P., and Durantel, D. (2016). HDV RNA replication is associated with HBV  
117 repression and interferon-stimulated genes induction in super-infected hepatocytes. *Antiviral Res* *136*,  
118 19-31. 10.1016/j.antiviral.2016.10.006.

119 Casey, J.L. (2012). Control of ADAR1 editing of hepatitis delta virus RNAs. *Curr Top Microbiol Immunol*  
120 *353*, 123-143. 10.1007/82\_2011\_146.

121 Dziri, S., Rodriguez, C., Gerber, A., Brichtler, S., Alloui, C., Roulot, D., Deny, P., Pawlotsky, J.M.,  
122 Gordien, E., and Le Gal, F. (2021). Variable In Vivo Hepatitis D Virus (HDV) RNA Editing Rates  
123 According to the HDV Genotype. *Viruses* *13*, 1572. 10.3390/v13081572.

124 Glenn, J.S., Watson, J.A., Havel, C.M., and White, J.M. (1992). Identification of a prenylation site in  
125 delta virus large antigen. *Science* *256*, 1331-1333. 10.1126/science.1598578.

126 Hindson, B.J., Ness, K.D., Masquelier, D.A., Belgrader, P., Heredia, N.J., Makarewicz, A.J., Bright, I.J.,  
127 Lucero, M.Y., Hiddessen, A.L., Legler, T.C., et al. (2011). High-throughput droplet digital PCR system  
128 for absolute quantitation of DNA copy number. *Anal Chem* *83*, 8604-8610. 10.1021/ac202028g.

129 Koh, C., Heller, T., and Glenn, J.S. (2019). Pathogenesis of and New Therapies for Hepatitis D.  
130 *Gastroenterology* *156*, 461-476 e461. 10.1053/j.gastro.2018.09.058.

131 Lempp, F.A., Schlund, F., Rieble, L., Nussbaum, L., Link, C., Zhang, Z., Ni, Y., and Urban, S. (2019).  
132 Recapitulation of HDV infection in a fully permissive hepatoma cell line allows efficient drug evaluation.  
133 *Nat Commun* *10*, 2265. 10.1038/s41467-019-10211-2.

134 Lucifora, J., and Delphin, M. (2020). Current knowledge on Hepatitis Delta Virus replication. *Antiviral*  
135 *Res* *179*, 104812. 10.1016/j.antiviral.2020.104812.

136 Sato, S., Cornillez-Ty, C., and Lazinski, D.W. (2004). By inhibiting replication, the large hepatitis delta  
137 antigen can indirectly regulate amber/W editing and its own expression. *J Virol* 78, 8120-8134.  
138 10.1128/JVI.78.15.8120-8134.2004.

139 Turon-Lagot, V., Saviano, A., Schuster, C., Baumert, T.F., and Verrier, E.R. (2020). Targeting the Host  
140 for New Therapeutic Perspectives in Hepatitis D. *J Clin Med* 9, 222. 10.3390/jcm9010222.

141

## 142 **Figure Legends**

143 **Figure 1: ddPCR assays for edited and non-edited HDV sequences.** (A-B) ddPCR duplex assays  
144 from (A) increasing amounts of non-edited or edited HDV templates or (B) mixed template with variable  
145 proportions of non-edited and edited sequences. Results are the means +/- SD of three independent  
146 assays. (C-F) HuH7.5-NTCP cells were transfected with the indicated siRNA 6 and 10 days after  
147 seeding. One day after the last transfection, cells were infected with HBV and HDV. At the indicated  
148 days post-infection (dpi), (C) intracellular HDAg and ADAR proteins were analyzed by western blot  
149 whereas the levels of intracellular (D) ADAR mRNAs, (E) HDV RNAs and (G) HBV RNAs were assessed  
150 by RT-qPCR analyses using *PRNP* mRNA for normalization. (F) The levels of HDV non edited and  
151 edited RNAs were analyzed by RT-ddPCR. Results are either presented as (upper graph) copies  
152 detected in the reaction or (lower graph) ratios of edited vs non edited HDV RNAs. (C-F) Results are  
153 the means +/-SD of two independent experiments each performed with 3 biological replicates.

154

155 **Figure 2: Loss of infectivity of HDV particles produced upon FTI.** dHepaRG cells or PHH from two  
156 donors were co-infected with HBV and HDV and treated or not 3 days later with FTI-277 (10  $\mu$ M) for 10  
157 days. Cells were collected and (A) the levels of intracellular HDV RNAs were analyzed by RT-qPCR  
158 using *PRNP* mRNA for normalization while (B) the editing rate was analyzed by RT-ddPCR. Supernatant  
159 were collected, concentrated by PEG precipitation and (C) the levels of extracellular HDV RNAs were  
160 assessed by qRT-PCR whereas (D) the editing rate was analyzed by RT-ddPCR. (E-F) Naïve HuH7.5-  
161 NTCP cells were infected with the different concentrated supernatants with 50 vge/cell (adjusted volume  
162 according to the initial concentration calculated in C). Six days later, (E) levels of intracellular HDV RNAs  
163 were assessed by RT-qPCR analyses using *PRNP* mRNA for normalization and (F) expression of HDAg  
164 was assessed by immunofluorescence analyses. (A-E) Results are expressed as ratios to mock treated  
165 cells and presented as mean +/- SD of at least three independent experiments for dHepaRG cells and  
166 two donors for PHH.

167 **Acknowledgements**

168 The authors declare no conflict of interest. The authors would like to thank Anaëlle Dubois and Jennifer  
169 Molle (CRCL, Lyon, France) for their help with the isolation of primary human hepatocytes, as well as  
170 the staff from Prof Michel Rivoire's surgery room for providing us with liver resection. We also thank Prof  
171 Fabien Zoulim and Dr Barbara Testoni (CRCL, Lyon, France) for the access to the QX200 AutoDG  
172 Droplet Digital PCR System. We thank Sarah Heintz and Anaëlle Dubois (CRCL, Lyon, France) for  
173 having made ddPCR procedures as easy as dice game rules, and Guillaume Fourier and Martial  
174 Saumier (Bio-Rad) for helpful technical expertise.

175

176 **Funding.**

177 This work of the Interdisciplinary Thematic Institute IMCBio, as part of the ITI 2021-2028 program of the  
178 University of Strasbourg, CNRS and Inserm, was supported by IdEx Unistra (ANR-10-IDEX-0002), and  
179 by SFRI-STRAT'US project (ANR 20-SFRI-0012) and EUR IMCBio (ANR-17-EURE-0023) under the  
180 framework of the French Investments for the Future Program. Eloi R. Verrier acknowledges fundings  
181 from Inserm, the Agence Nationale de Recherches sur le Sida et les Hépatites Virales (ANRS, grant  
182 number ECTZ104527), and the French National Research Agency (ANR, grant number ANR-21-CE15-  
183 0035-01 DELTArget). Julie Lucifora acknowledges fundings from Inserm, CNRS, University of Lyon,  
184 and ANRS (grant number ECTZ102776).

## **Loss of hepatitis D virus infectivity upon Farnesyl transferase inhibitor treatment associates with increasing RNA editing rates revealed by a new RT-ddPCR method**

### **Declaration of interest statement**

The authors declare no conflict of interest.

## **Loss of hepatitis D virus infectivity upon Farnesyl transferase inhibitor treatment associates with increasing RNA editing rates revealed by a new RT-ddPCR method**

### **Highlights**

- We setup an innovative RT-ddPCR method for the quantification of hepatitis D virus RNA
- This method allows the discrimination of edited and non-edited versions of the viral genome
- We demonstrated that farnesyl transferase inhibitor treatment of infected cells led to an increase in intracellular HDV RNA
- FTI treatment induced an accumulation of edited HDV genomes, both intracellularly and in the supernatant of infected cells
- This accumulation is associated to a decrease in the infectivity of HDV viral particles

Figure 1

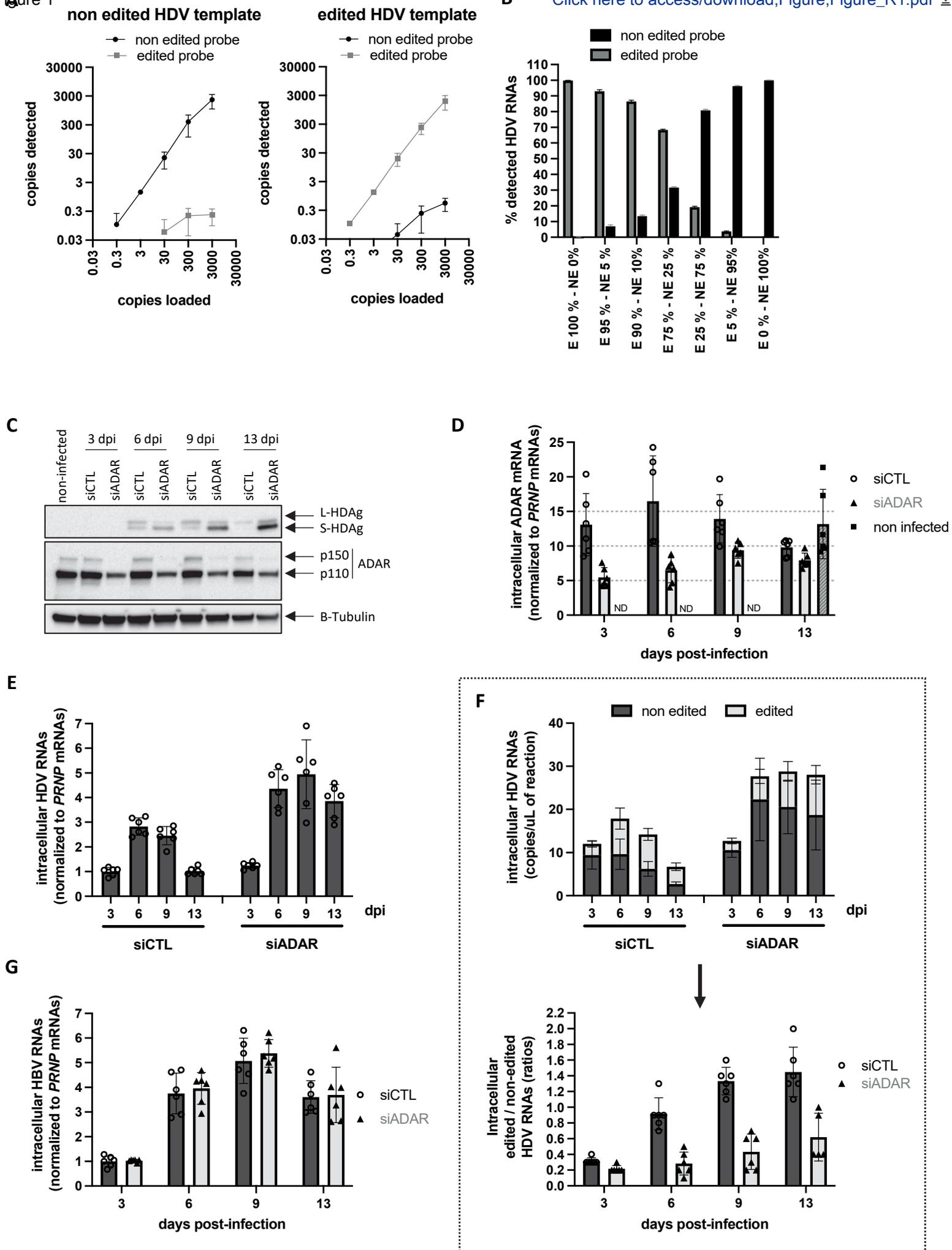
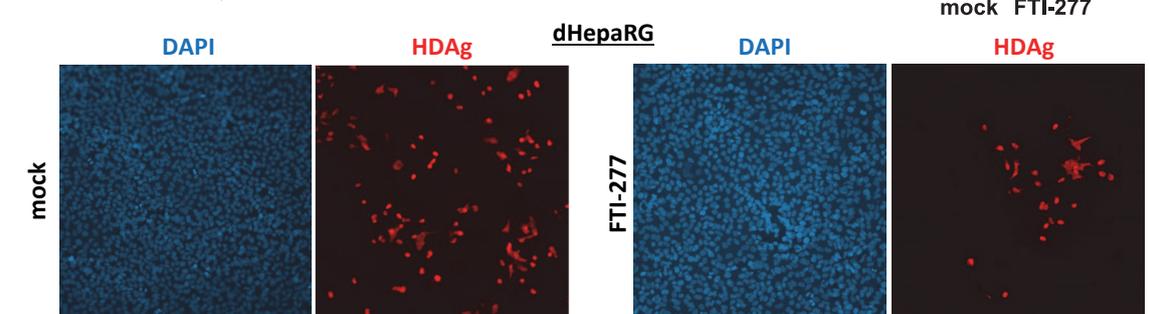
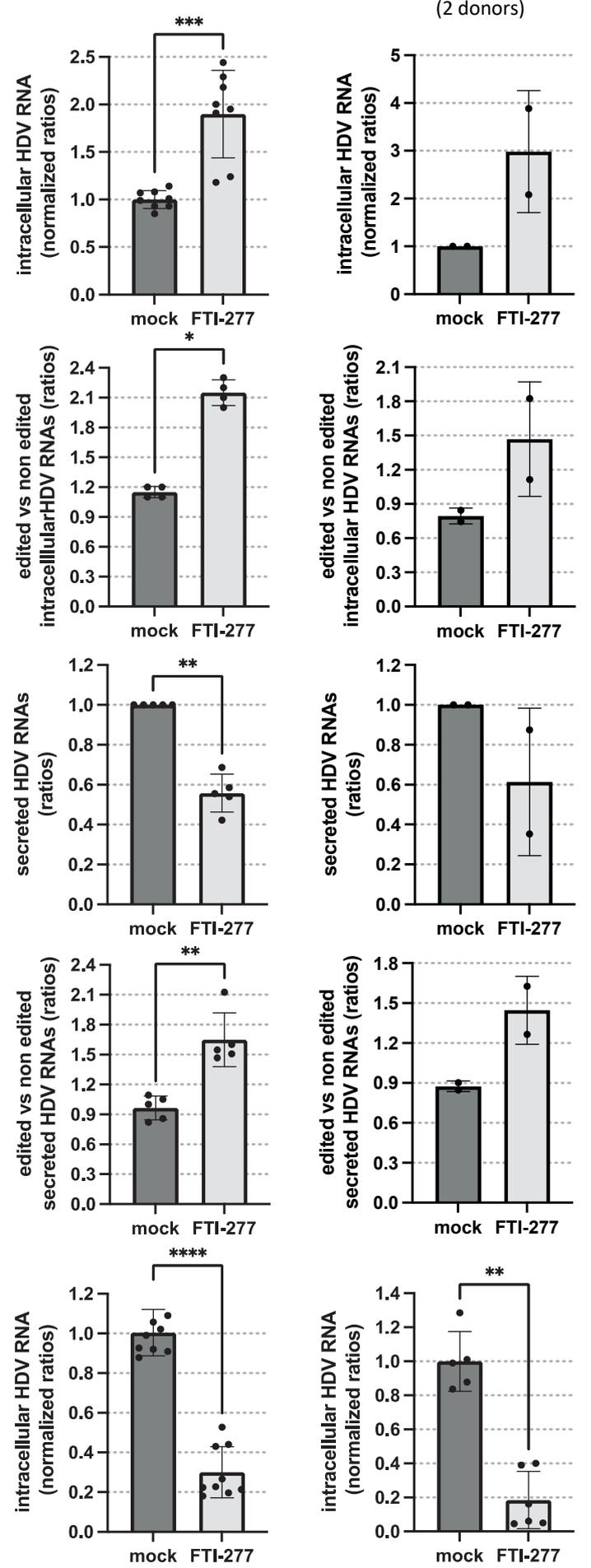
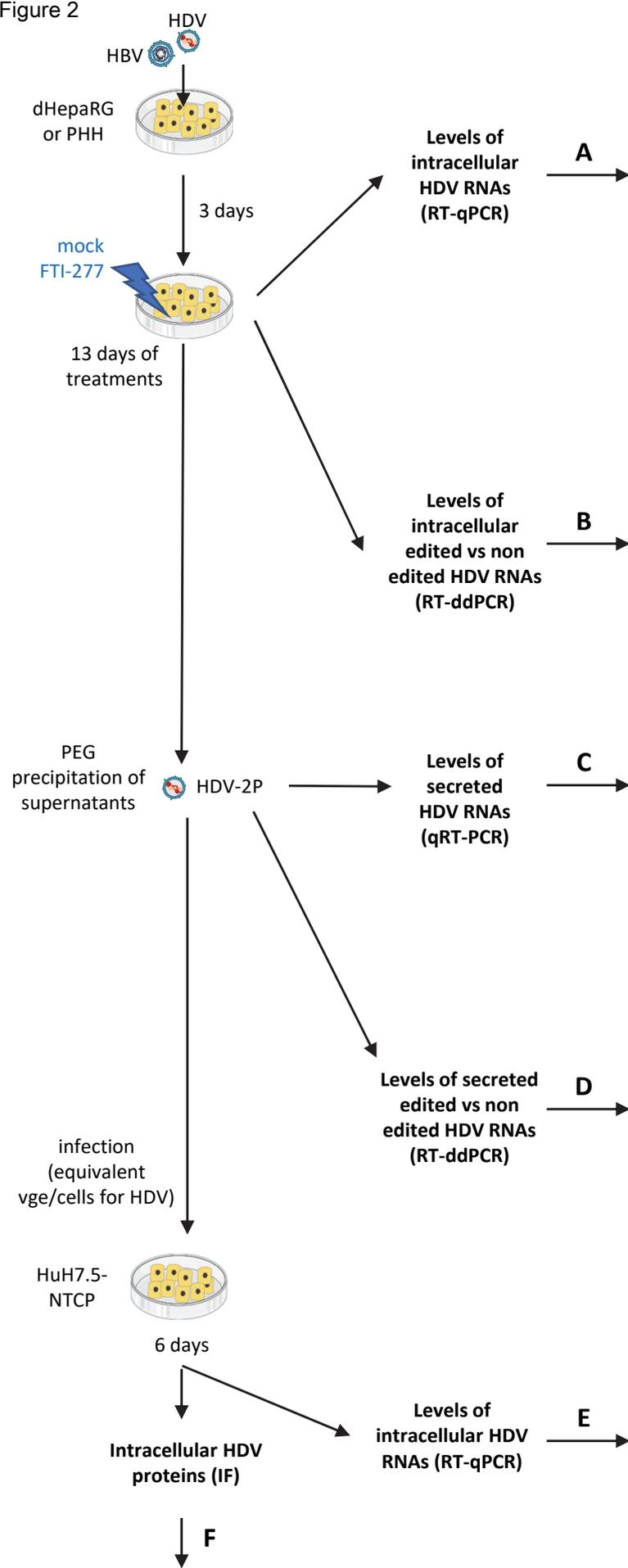


Figure 2





[Click here to access/download](#)

**Supplementary Material**

Verrier-Lucifora\_Supplementary Material-R1-V4.pdf

