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1 **HCV Core protein needs triacylglycerols to fold onto the**
2 **endoplasmic reticulum membrane**

3

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33

34 **Abstract.**

35 Lipid droplets (LDs) are involved in viral infections, but exactly how remains unclear. Here, we
36 study the hepatitis C virus (HCV) whose Core capsid protein binds to LDs but is also involved
37 in the assembly of virions at the endoplasmic reticulum (ER) bilayer. We found that the
38 amphipathic helix-containing domain of Core, D2, senses triglycerides (TGs) rather than LDs
39 *per se*. In the absence of LDs, D2 can bind to the ER membrane but only if TG molecules are
40 present in the bilayer. Accordingly, the pharmacological inhibition of the diacylglycerol O-
41 acyltransferase enzymes, mediating TG synthesis in the ER, inhibits D2 association with the
42 bilayer. We found that TG molecules enable D2 to fold into alpha helices. Sequence analysis
43 reveals that D2 resembles the apoE lipid-binding region. Our data support that TG in LDs
44 promotes the folding of Core, which subsequently relocalizes to contiguous ER regions. During
45 this motion, Core may carry TG molecules to these regions where HCV lipovirions likely
46 assemble. Consistent with this model, the inhibition of Arf1/COPI, which decreases LD surface
47 accessibility to proteins and ER-LD material exchange, severely impedes the assembly of
48 virions. Altogether, our data uncover a critical function of TG in the folding of Core and HCV
49 replication and reveals, more broadly, how TG accumulation in the ER may provoke the
50 binding of soluble amphipathic helix-containing proteins to the ER bilayer.

51

52

53

54 **Introduction**

55 Lipid droplets (LDs) regulate intracellular lipid storage and secretion via lipoproteins [1].
56 Pathogens or viruses often hijack these functions to replicate [1–4]. Indeed, viral infections
57 often correlate with accentuated LD biogenesis or catabolism [1]: this is the case with many
58 flaviviruses such as Dengue or Zika [5–9], hepacivirus such as hepatitis C (HCV) [10–13],
59 enteroviruses such as polioviruses, herpes simplex virus type 1 (HSV-1) [8,14], or the severe
60 acute respiratory syndrome coronavirus 2 [15,16]. How viruses interfere with the LD lifecycle
61 is poorly understood.

62

63 LDs resemble lipoproteins or chylomicrons: they have a neutral lipid core composed of
64 triacylglycerols (TG) and sterol esters, surrounded by a phospholipid monolayer containing
65 proteins [17]. Many proteins associated with LDs have binding motifs found in apolipoproteins
66 [18], such as amphipathic helices (AHs). Like lipoproteins, LD biogenesis occurs in the
67 endoplasmic reticulum (ER) bilayer, yet, *via* a different mechanism [19]. Most LDs emerge into
68 the cytosol [17,20] while lipoproteins assemble in the ER lumen [19].

69

70 The LD lifecycle starts with the synthesis of neutral lipids. For example, diacylglycerol O-
71 acyltransferase 1 and 2 (DGAT1,2) generates TG, which is released in the hydrophobic core
72 of the ER bilayer [21]. TGs are then freely mobile [22] but can condense to nucleate an LD that
73 buds off into the cytosol and likely detaches from the bilayer [23]. At this stage, individual TGs
74 may remain in the bilayer but at a concentration below the critical condensation concentration
75 [24]. The cytosolic LD is tethered to the ER membrane by several factors such as Rab18
76 [25,26]. Following the action of the Arf1/COPI machinery, a subset of mature LDs reconnects
77 to the ER, and the organelles may subsequently exchange lipids and proteins [23,27–29].
78 Finally, LD catabolism happens by the hydrolysis of TG by lipases such as adipose triglyceride
79 lipase ATGL or lipophagy [30]. Fatty acids released during these processes are used for
80 energy or making membranes or are re-esterified by DGAT1 to prevent lipotoxicity [31–33].
81 HCV viral proteins interfere with each of these steps of the LD lifecycle to promote efficient
82 virus replication and secretion [11,12,34].

83

84 HCV remains a significant cause of chronic hepatitis, liver cirrhosis, and hepatocellular
85 carcinoma [10]. It has a positive single-stranded RNA of 9.6 kb encoding a polyprotein
86 precursor of ~3,000 amino acids. In infected liver cells, the polyprotein is processed by several
87 viral and cellular proteases into ten proteins [35,36]: structural proteins (Core, E1, E2), which
88 represent the major constituents of the viral particle, and non-structural proteins (p7, NS2,
89 NS3, NS4A, NS4B, NS5A, NS5B). Among these, the Core protein of the capsid and NS5A
90 localize to the LDs' surface using AH motifs [37,38]. For the JFH1 HCV, the binding of Core to

91 LDs is critical for the production of HCV virions [39,40], often referred to as 'lipovirions'
92 harboring neutral lipids as well as apolipoprotein B or E [37,41]. The exact functions of LDs in
93 the assembly and secretion of HCV virions are still elusive.

94

95 Core is a 191 amino acid-long peptide harboring (Figure 1A) an N-terminal hydrophilic D1
96 domain, a D2 domain of 42 amino acids containing two AHs bridged by a small disordered
97 loop (Figure 1B), and a transmembrane D3 domain of 20 amino acids at the C-terminal, serving
98 as a signal peptide [35,42]. This immature form of Core is anchored to the ER bilayer by D3.
99 Cleavage of D3 by the cellular Signal Peptide Peptidase (SPP) yields mature Core, made by
100 D1D2 (Figure 1A), which localizes to LDs by D2 [10]. Intriguingly, D1D2 needs to associate
101 with the ER membrane to participate in the virions' assembly [43–45]. It is unknown if D1D2
102 binds directly to the ER and/or traffics between LDs to the ER bilayer to assemble virions. We
103 sought to address this question in this paper.

104

105 **Results**

106 **Mature Core and D2 preferentially bind to LDs over the ER bilayer.**

107 Core is translated at the N-terminus of the viral polyprotein and released after two successive
108 cleavages by cellular proteases. The first cleavage generates an immature Core, D1D2D3
109 (Figure 1A). The second cleavage, just before D3, releases mature Core D1D2 (hereafter
110 Core) from the ER bilayer. D1D2 binds to LDs by the AHs of D2 (Figure 1B) [38,39,42].

111
112 To investigate the localization of Core to LDs vs. ER, we used the hepatocarcinoma Huh7
113 model cell line and co-expressed mCherry-sec61 β , an ER marker, with JFH1-D1D2D3. Next,
114 the cells were fed with oleic acid (OA) for 24hr to induce LDs, then fixed and immunostained
115 (IF) for Core. Core was on all LDs as previously shown [3,39,46] but did not display a
116 noticeable ER localization (Figure 1C). This observation indicates that cleavage at D3
117 occurred, and Core, i.e., D1D2, was released but incapable of binding to the ER bilayer, at
118 least not as efficiently as to LDs.

119
120 To confirm Core localization, we expressed D1D2 directly instead of D1D2D3 but could not
121 detect any protein signal by IF. D1D2 is highly prone to proteasomal degradation [47]. When
122 we treated the cells with the protease inhibitor MG132, before D1D2 overexpression, we found
123 by IF that D1D2 was in the nucleus (Figure 1D, Figure S1A). Such nuclear localization is
124 mediated by interaction with the importin-PA28 complex and signatures Core's degradation
125 [47]. Indeed, several nuclear localization signals were previously identified in D1 [48].

126
127 Together, the above results support the model that when cleavage happens at D3, a fraction
128 of Core, which is soluble, is stabilized by LDs and escapes degradation into the nucleus. The
129 discrepant localization of Core upon the expression of D1D2D3 and D1D2 likely pertains to
130 kinetics reasons. In D1D2, the protein is directly released in the cytosol and imported to the
131 nucleus. In D1D2D3, a fraction of Core could also be released and imported to the nucleus.
132 However, the initial docking of the protein to the ER bilayer, close to LDs, may facilitate its
133 rapid binding and stabilization to LDs.

134
135 To get more insights into Core's localization, we focused on D2, missing the nuclear import
136 signals present in D1. eGFP-D2 displayed a localization similar to Core: it was cytosolic,
137 localized to all LDs but not to the ER bilayer (Figure 1E, Figure S1B). This observation was
138 confirmed in HeLa cells, which have fewer or no LDs in non-fed conditions: eGFP-D2 was
139 soluble and sometimes presented aggregates; in OA feeding, it was on LDs and did not show
140 a noticeable ER signal (Figure S1C).

141

142 Finally, to better visualize the ER membrane and know whether D2 binds to it, we added a
143 hypotonic culture medium to the cells (Figure 1F) to induce the formation of Giant intracellular
144 ER vesicles (GERVs) [22,49,50], offering a higher spatial resolution (Figure 1F). We
145 transfected the Huh7 cells with eGFP-D2 and ER-BFP, marking the ER lumen, and induced
146 intracellular GERVs (Figure 1G, Figure S1D). We found a clear D2 signal around LDs but not
147 on the surface of the intracellular GERVs, agreeing with our above findings that D2, and likely
148 Core, cannot directly bind to the ER membrane. Contrasting with this behavior, when we
149 repeated the experiment with eGFP-Plin1, we found a signal on both intracellular GERVs and
150 LDs [51] (Figure 1H, Figure S1E), albeit a higher signal was on the latter.

151

152 **D2 preferentially folds onto droplets deficient in phospholipids**

153 We used a reductionist approach to validate our findings. Within a single glass chamber, we
154 added both TG-in-buffer droplets and giant unilamellar vesicles (GUVs) made of dioleoyl
155 phosphatidylcholine (DOPC) and dioleoyl phosphatidylethanolamine (DOPE), at a ratio of
156 70/30. We then injected purified JFH1-eGFP-D2 and found its exclusive recruitment to the
157 droplets, not the GUV bilayers (Figure 2A,2C). This behavior contrasted with the rhodamine-
158 tagged AH of NS5A-HCV that was efficiently bound to both droplets and bilayers (Figure
159 2B,2C). The less-selective binding of the NS5A-AH likely pertains to its multiple bulky
160 hydrophobic residues [52], compared with D2 (Figure S2A).

161

162 To confirm that the folding of D2 AHs mediated the binding to the TG/buffer interface, we used
163 circular dichroism to determine the presence of alpha-helices. We prepared TG-in-buffer
164 droplets by bath sonication in the presence of 0.1 DOPC in TG (w/w to TG); in parallel, we
165 prepared DOPC liposomes by ultra-power sonication, yielding nanometric vesicles. We then
166 used a synthesized J6-D2 peptide and performed circular dichroism experiments with both
167 solutions. No alpha helix signal was noticed when the peptide was alone in the buffer (Figure
168 2D,2E). Alpha-helices' signal appeared in the presence of droplets (Figure 2D) unless these
169 were highly covered with phospholipids (Figure S2B).

170 In contrast, alpha-helices were barely detectable with liposomes, independently of liposome
171 size (Figure 2E, S2C). This data supports that D2 folds better when TG is exposed, which
172 agrees with the above *in vitro* and cellular studies. Photobleaching experiment of D2 on the
173 TG-in-buffer droplets showed a slow recovery, indicating that D2 firmly folds onto this interface
174 (Figure 2F, Figure S2D).

175

176 Since by circular dichroism we found that the phospholipid level influenced D2 folding (Figure
177 2D, S2B), we probed by fluorescence how the monolayer phospholipid density impacts D2
178 binding to the model LDs. We made artificial TG-in-buffer droplets covered by different DOPC

179 levels, reported by the rhodamine-DOPE (Rhod-PE) used at 1% (w/w to DOPC) and serving
180 as a phospholipid marker (Figure 2G, S2E). The droplets were then introduced in a chamber,
181 and purified JFH1-eGFP-D2 was added. We observed a non-uniform signal distribution
182 between droplets (Figure 2H). D2 associated better with droplets containing less DOPC,
183 providing more access to TG (Figure S2E, S2F, Figure 2I). This restriction of binding by DOPC
184 was more drastic on D2 than the AH of Caveolin1 [53]. Therefore, the LD phospholipid
185 coverage can finely tune D2 binding; a tight cover will prevent D2 binding despite its strong
186 association with bare TG/water interface (Figure 2I), agreeing with the circular dichroism
187 results (Figure S2B).

188

189 Taken together, our results indicate that Core can bind alone to LDs and not to the ER bilayer.
190 The AHs of D2 fold and remain firmly attached to TG/aqueous interfaces, and this binding
191 capacity is dependent on the phospholipid monolayer density.

192

193 **D2 competes off Perilipin 3 and not 2 for associating with LDs**

194 Like phospholipids, proteins on LDs can also have a barrier function and prevent the non-
195 specific recruitment of proteins [54,55]. In the Huh7.5 liver cells, perilipin (Plin) 2 and 3 are the
196 most abundant LD protein coats and Plin1 is not expressed [56]. When these cells were
197 transfected with mCherry-Plin1-3, the proteins perfectly localized to the LD surface (Figure
198 S3A). To test whether they interfere with D2 binding, we did a competition assay wherein JFH1-
199 eGFP-D2 is co-expressed with the mCherry-Plins [51,55]. After 24h of co-transfection and OA
200 supply, the cells were visualized under the same imaging settings (Figure 3A). We took cells
201 presenting similar D2 and Plin levels based on their bulk signals. Then, we determined the
202 protein fluorescence intensity on the LDs and subtracted the surrounding background signal.
203 The highest protein signal was then set to 1, to compare better the relative recruitment of D2
204 vs. Plins.

205

206 We found that D2 almost systematically excluded Plin3 from the LDs' surface under our
207 working conditions (Figure 3A-C) (24hr after OA loading); this exclusion was neat on nuclear
208 LDs (Figure 3A, arrowheads), presenting fewer LDs and a lower Plin3 background. D2
209 colocalized with Plin2 on LDs, and there was no noticeable exclusion between the two proteins
210 (Figure 3A-C), even on nuclear LDs (Figure 3A, arrowhead). Finally, Plin1, which is absent in
211 the liver and has a higher binding affinity to LDs than Plin2,3 [51], greatly displaced D2 from
212 LDs (Figure 3A-C). This binding exclusion of Plin1 was validated *in vitro* by the eviction of D2
213 by Plin1 from shrinking TG-buffer interfaces (Figure S3B, S3C) [55]. This observation was also
214 true for JFH1-Core, which was displaced from LDs by Plin1 (Figure S3D).

215

216 D2 firmly bound the bare TG/buffer interface *in vitro* (Figure 2F, Figure S2D). To get insights
217 into its binding stability to LDs, we performed photobleaching experiments. We found different
218 recovery kinetics between LDs from different cells highly expressing D2 (Figure 3D, S3E): the
219 majority of LDs had a rapid characteristic recovery time (<20sec), and few LDs recovered more
220 slowly (> 20sec) (Figure 3E). eGFP is soluble, bigger than D2, and could provoke the rapid
221 fall-off of D2. To rule this out, we designed constructs where eGFP is bridged to D2 by flexible
222 glycine linkers, eGFP-GGGGS-D2 and eGFP-(GGGGS)₂-D2. These proteins displayed similar
223 behavior to eGFP-D2, i.e., cytosolic and recovered rapidly on most LDs (Figure S3F, S3G).
224 This observation supports that eGFP was not responsible for the rapid dissociation of D2 from
225 LDs. Lastly, even within a single cell, D2 showed different recovery rates between LDs (Figure
226 3F, Movie), indicating that an LD subset provides a more stable binding to D2.

227

228 In conclusion, the above data support that Core can alter the proteome of LDs and, possibly,
229 LD functions. Strongly LD-associating proteins can compete and prevent D2 from accessing
230 TG. Finally, a subpopulation of LDs more than others appears to better stabilize D2.

231

232 **Arf1/COPI inhibition decreases Core recruitment to LDs and lipoviroparticles.**

233 Our above findings show that the surface coverage level of LDs modulates the binding of
234 Core/D2, which more stably occurred on an LD subset. Interestingly, the Arf1/COPI machinery,
235 involved in the budding of vesicles from the Golgi apparatus for retrograde cargo trafficking,
236 acts also on an LD subpopulation; it modulates the surface accessibility of these LDs to
237 proteins [23,27]. For instance, the inhibition of Arf1/COPI impedes the binding to LDs of ATGL
238 [57,58], GPAT4 [23], Plin2 or ADRP, but not Plin3 or TIP47 [57]. Thus, we probed whether
239 Arf1/COPI inhibition prevents Core binding to LDs.

240

241 To block Arf1/COPI action, we used Brefeldin A (BFA), which inhibits the activity of the guanine
242 exchange factor of Arf1, GBF1, and the assembly of the machinery. We loaded Huh7 cells with
243 OA for 24hr to increase LDs, treated them with BFA, and transfected them with Core-D1D2D3
244 before imaging, after 24hr. Compared to control cells, BFA decreased Core association with
245 LDs and promoted localization to the nucleus (Figure 4A-B, S4A), reminiscent of D1D2
246 localization when directly expressed (Figure 1D). This nuclear localization probably arose from
247 the redirection of Core from LDs or the ER to the nucleus where it is degraded [47]. We also
248 observed another phenotype where some LDs had a complete Core signal, few others
249 completely lacked it, while others displayed arc signals (Figure 4A, Figure S4A-B), as similarly
250 seen previously [45]. Small and medium LDs, <2µm in size, were more impacted than larger
251 ones (Figure 4C). The arc feature may reveal the onset of LD-ER or LD-Golgi contacts
252 excluding Core [59]; alternatively, it could be the signature of LDs that did not fully emerge into

253 the cytosol [20], as seen with the appearance of apoB crescent shapes when apoB is
254 overexpressed in Huh7 hepatocyte cells [60]. In summary, the activity of GBF1 is necessary
255 for the correct binding of Core to LDs and its non-degradation.

256

257 We repeated the above experiment with D2 and obtained the same phenotype (Figure 4D).
258 Within a single cell, D2 was on an LD subset, or displayed arc features, or was utterly absent
259 from LDs. The arc and ring signals of D2 recovered very rapidly following photobleaching,
260 showing that such signals did not correspond to a firm binding to the LDs (Figure S4C). We
261 also observed other phenotypes wherein D2 is absent from LDs and, instead, formed puncta
262 in the cytosol. This phenotype is also reminiscent of mature Core's nuclear localization in BFA
263 treatment (Figure 4A, S4A). Indeed, since D2 lacks D1, it could not be targeted to the nucleus
264 for degradation but was prone to aggregation. As D2 is a basic soluble peptide, unlikely to
265 possess any biological function, the above observations support that BFA treatment decreased
266 the LD surface accessibility to proteins.

267

268 Since BFA principally interferes with GBF1, which activates Arf1, we decided to target the
269 COPI coatomers. We used siRNAs against the β COP subunit. We transfected JFH1-Core in
270 Huh7 cells pre-treated with the siRNAs for 24hr (Figure 4E, Figure S4D-E). The impact of the
271 siRNA treatment was noticeable through the loss of the compactness of the Golgi, which was
272 more fragmented (Figure S4F). Similar to previously shown, the reduction of β COPI decreased
273 the levels of Core [61] (and also ADRP [57]) (Figure S4E) but, consistent with our hypothesis,
274 it led to impaired binding of Core to LDs and yielded phenotypes similar to BFA (Figure 4E-
275 F, S4D-F) [45].

276

277 Since we found that Arf1/COPI inhibition decreased Core association with LDs, we wondered
278 how this would impact the production of lipoviral particles. We infected Huh7 cells with Jc1
279 HCV chimera and analyzed Core localization by IF, with or without OA addition (Figure S4G).
280 Core was barely found around LDs in the absence of OA (Figure S4H). Instead, LDs were
281 more extensive when OA was added and they had a clear Core signal at their surface (Figure
282 S4H). This observation suggests that when more TGs are made, Core has higher chances to
283 interact with LDs. Under the same infection and OA feeding conditions, if Plin1, in contrast to
284 Plin2 or 3, was overexpressed to mask the LD surface, Jc1-Core binding to LDs was drastically
285 diminished (Figure S4I); this result agrees with our previous competition experiments (Figure
286 3B).

287

288 Next, we analyzed the intracellular and extracellular lipoviral particles made at 24h post-
289 infection. Shortly after infection, without OA, the infectious virions were mainly found

290 intracellularly (300 FFU/mL versus less than 100 FFU/mL for the extracellular infectious
291 virions) (Figure S4J). On the opposite, in OA-treated cells, the amount of intracellular infectious
292 particles was negligible (less than 20FFU/mL) compared with the extracellular one (500
293 FFU/mL) (Figure S4J). This data indicates that secretion of HCV particles was highly
294 stimulated when TG biosynthesis is stimulated by OA addition. It highlights the importance of
295 TG for an efficient virion secretion.

296

297 Finally, we determined the impact of Arf1/COPI in the OA feeding condition. At eight hours
298 post-infection, cells were treated with BFA for 16hrs and then fixed (Figure 4G). Core was
299 found predominantly on LDs' surface in control cells but much less in BFA-treated ones (Figure
300 4H). In the latter, we found a drastic reduction in the number of assembled virions, both
301 intracellularly and extracellularly, below the detection threshold, respectively, of 50 and 10
302 FFU/mL (Figure 4I). Finally, washing out BFA led to the relocalization of Core to LDs and the
303 efficient re-assembly of lipoviral particles (Figure S4K-L), confirming the crucial role of
304 Arf1/COPI in the assembly of HCV virions.

305

306 **Triacylglycerols recruit D2 to the ER membrane**

307 Core is required for the assembly of lipoviral particles that takes place at the ER membrane. A
308 question is how the binding of Core to LDs relates to this assembly process at the ER bilayer.
309 Our above results showed that D2 associates firmly with TG/water interfaces, on LDs.
310 However, the ER membrane, the biosynthesis site of neutral lipids, can also bear TG
311 molecules, especially when LD assembly is impaired or delayed [22,62,63]. Thus, we
312 hypothesized that the presence of TG could promote D2 binding to the ER bilayer. Such
313 binding would guarantee Core's presence at the ER for the assembly of virions. To test our
314 hypothesis, we worked with intracellular giant ER vesicles (GERVs) to gain spatial resolution
315 of the ER membrane, and TG and D2 localization [50]. We chose Cos7 cells because they
316 barely present LDs, unless they are cultured in an energy-rich media, and we can monitor TG
317 biosynthesis [22] and D2 localization (Figure 1F-H).

318

319 We transfected the cells with eGFP-D2 and induced intracellular GERVs, marked by RFP-
320 KDEL (Figure 5A, S5A-B) or BFP-ER (Figure 5B). Consistent with our data in Figure 1G, D2
321 was absent from the intracellular GERVs' surface and was cytosolic (Figure 5A-B, Figure S5A-
322 B). Then, we fed the cells with OA, supplemented with OA-C12-BODIPY, to induce the
323 biosynthesis of fluorescent TG molecules and their accumulation into the GERVs' membranes
324 [22]. To also report for the GERVs' membrane hydrophobicity by the presence of neutral lipids,
325 we used LipidTox in the system [22] (Figure 5A, S5C). We observed an OA signal in GERVs,
326 likely fluorescent TG, which was strikingly concomitant with a clear binding of D2 (Figure 5A-

327 B, S5A-B). The level of D2 on the neutral lipid-rich intracellular GERVs increased with the
328 LipidTox signal (Figure 5C, S5C). These results strongly agree with our model that the binding
329 of D2 to GERVs is promoted by the biosynthesis and accumulation of TG in the bilayer (Figure
330 5D).

331
332 To ensure that the localization of D2 to the GERVs' membrane was due to TG accumulation
333 within the bilayer (Figure 5D), we repeated the experiment but with pre-treating the cells with
334 DGAT1,2 inhibitors to block TG synthesis (Figure S5D-G). Under this condition, when the
335 intracellular GERVs were fed with OA, as previously, the LipidTox GERV signal was barely
336 visible and the binding of D2 to the GERVs was severely impaired (Figure 5E-F, Figure S5E-
337 G). This data also support that the presence of TG in the ER bilayer mediated the recruitment
338 of D2 to the GERV bilayers.

339
340 Next, we did photobleaching experiments of D2 on GERVs containing TG. On randomly picked
341 GERVs from different cells, the D2 recovery rate was lower than on cellular LDs (Figure 5G-
342 H, S5I), but it was still faster than on bare TG/buffer interfaces (Figure 2F). We interpret this
343 data as if the protein coverage of the GERVs was sparser than on LDs, possibly due of the
344 swelling process. As a consequence, TG was more exposed and accessible to D2 on these
345 GERVs. Consistent with this interpretation, when we overexpressed mCherry-sec61 β , an
346 integral ER membrane protein, instead of mCherry-KDEL or ER-BFP, which mark the ER
347 lumen, the binding of D2 to GERVs was significantly reduced despite the OA addition (Figure
348 5I). The lower the mCherry-Sec61 β on GERVs, the higher the eGFP-D2 signal (Figure 5J,
349 Figure S5J); GERVs with higher LipidTox signals still recruited more D2 (Figure S5J). Based
350 on this observation, we concluded that mCherry-Sec61 β competed off D2 from GERVs,
351 exactly as Plin1 did on LDs (Figure 3B).

352
353 Altogether, these data indicate that TG molecules, independently of their organelle localization,
354 can recruit Core. They seem to stabilize the binding of Core to the ER membrane, which is a
355 necessary condition for the assembly of lipoviral particles.

356
357 **D2 binds to bilayer membranes physically connected to TG droplets**
358 Although TG molecules in GERVs favored D2 recruitment, it could be via indirect mechanisms.
359 To directly test our model that TG molecules infused in the ER bilayer can recruit D2, we turned
360 to *in vitro* reconstitution with droplet-embedded vesicles (DEVs) [64]. DEVs consist of a neutral
361 lipid droplet embedded in a giant unilamellar vesicle (GUV) [65], here made of DOPC:DOPE
362 (70: 30) and triolein (Figure 6A). We used rhodamine-PE to visualize the bilayer and monolayer
363 interfaces. Next, we added eGFP-D2 to the DEV solution and followed its localization. D2 was

364 recruited to the TG droplet and not the bilayer (Figure 6A, S6A), in contrast to NS5A-AH that
365 bound to both (Figure S6B). A droplet monolayer has a lower phospholipid packing than a
366 bilayer, even though they are in contiguity [53,66]. This discrepancy might explain the exclusive
367 recruitment of D2 to the monolayer (Figure 6A).

368

369 When the surface tension of DEVs is increased, TG molecules partition from the droplet to the
370 bilayer [67,68]. We used this strategy to increase TG levels in the bilayer and determine if this
371 would favor D2 recruitment to the bilayer, as seen with GERVs. After D2 bound to the droplet
372 of DEVs, we increased the bilayer tension by adding water to the bulk medium (Figure 6B) to
373 provoke the system's swelling and the delivery of TG to the bilayer [68]. In the course of
374 swelling, reported by the increase in the DEV radius, D2 increasingly bound to the DEV bilayer
375 region (Figure 6B). Thus, TG molecules released by the droplet to the bilayer likely recruited
376 D2. As a control, we induced the swelling of a bare GUV and we did not observe the
377 recruitment of D2 to the bilayer (Figure 6C), even if the latter is stretched to its maximum level.
378 In the DEV swelling experiment, the bilayer-localized D2 might come from the direct binding of
379 D2 from the bulk solution to the bilayer or the lateral diffusion of D2 from the droplet surface to
380 the bilayer. In the latter case, D2 could carry TG from the droplet surface to the bilayer.

381

382 Collectively, our *in vitro* results with DEVs and cell data with GERVs indicate that TG molecules
383 in the bilayer recruit and stabilize Core/D2 to the ER membrane.

384

385 **D2 has sequence similarities with apoE and a preference for TG**

386 Based on our above observations, we hypothesized that D2 has an affinity for TG. Since Core
387 assembles into lipoviral particles containing ApoE or ApoB [37,69], we asked whether D2
388 shares features with apolipoproteins. Sequence alignments of Core with apolipoproteins
389 revealed conserved regions between JFH1-Core and ApoE. Namely, we found conserved
390 regions between Core-D1 and ApoE (Figure S6C, S6D) and more substantial similarity
391 between Core-D2 and the lipid-binding domain of ApoE [18], i.e., the hydrophobic loop and
392 portion of their AHs involved in their association with TG/buffer interfaces (Figure 6D).
393 Phylogenetic tree analysis shows that D2, in contrast to the 11-mer repeats of perilipins,
394 clusters with apoE, apoC1, and the non-structural protein 1 of Dengue virus (Figure S6E), all
395 binding to TG/water interfaces. Based on this analysis, we decided to test whether D2 prefers
396 TG as compared with apoE.

397

398 To study the affinity of D2 for TG, we used a tensiometer approach [51,70]. We generated oil-
399 in-water droplets made either of TG or squalene, an intermediate neutral lipid in sterol
400 biosynthesis, and added the same concentration of J6-D2. Recruitment of D2 would be

401 accompanied by a decrease in surface tension to an equilibrium value (Figure 6E) [51,70]. We
402 found a reduction of tension that was much more striking for TG, from ~34 to ~11 mN/m, than
403 for squalene, from ~22mN/m to ~20mN/m, indicating that D2 was barely recruited to the
404 squalene/buffer interface (Figure 6F, 6G). We looked at the behavior of apoE, and it decreased
405 TG's surface tension as efficiently as for squalene (Figure 6G, S6F). These observations
406 support that D2 has a higher affinity for TG than for squalene and, also, a higher capacity to
407 distinguish the neutral lipid/water interfaces than apoE. In contrast to D2, apoE does not only
408 have the lipid-binding domain but also possesses multiple AHs forming a four-helix bundle that
409 unzips and associates with membranes [18] (Figure S6C). These AHs might induce the strong
410 binding of ApoE to the neutral lipid/water interface, regardless of the neutral lipid chemistry or
411 composition.

412

413

414

415 **Discussion**

416 The binding of Core to LDs facilitates the assembly of HCV viral particles. However, this
417 assembly occurs at the ER membrane bilayer and implicates also Core. Thus, Core protein
418 has to relocalize from LDs to the ER bilayer or bind directly to the latter. It is unknown how
419 such traffic happens.

420

421 We found that Core's LD-binding domain, D2, shares sequence similarities with the lipid-
422 binding part of apoE and likely has an affinity for TG. We propose that mature Core stably
423 binds to the surface of a subset of LDs from which it relocalizes to ER regions where virions
424 assemble (Figure 7A). This relocation might be assisted by other HCV viral proteins such as
425 p7-NS2 [46]. If LDs are absent or physically disconnected from the ER, Core may still localize
426 itself to the ER but would require the presence of TG in the membrane (Figure 7B). Such an
427 ER targeting mechanism appears so far uncommon for AH-containing proteins commonly
428 binding to LDs. An exception might Plin3, which may associate with the ER bilayer when
429 diacylglycerol levels in the membrane increases, for example, during the early steps of LD
430 biogenesis [71]. All these observations highlight a possible role for ER membrane-infused
431 neutral lipids in recruiting soluble proteins to the membrane.

432

433 Why does Core bind to LDs and not the ER, following the SPP-mediated cleavage at the D2-
434 D3 region? A first answer is that the free TG concentration in the ER should be low under
435 normal conditions, since most TGs are packaged into LDs. Thus, Core may not be able to
436 directly bind the ER, as we found in Figure 1,5. Since LDs are less packed with phospholipids
437 than the ER bilayer [53], their hydrophobic TG core is more accessible to soluble proteins and
438 allows the efficient folding of D2 onto LDs (Figure 2, S2). In this picture, the LD surface acts
439 as a protein folding partner for AHs and induces Core interfacial stabilization (Figure 7A). A
440 second answer is that the stable binding of Core to LDs prevents its nuclear import and
441 subsequent degradation (Figure 7A). In this view, LDs safeguard Core for degradation. In the
442 meantime, LD-bound Core serves as encapsidation machinery of the viral HCV RNA
443 [43,44,72–74]. Lastly, during the assembly of HCV particles, Core may stably move from LDs
444 to the ER bilayer, through ER-LD bridges [29,75], and carry TGs from the LD to the nascent
445 virion assembly site (Figure 7A). In this scheme, LDs would serve as a TG seed used by Core
446 for the assembly of virions.

447

448 In the absence of LDs, or when LDs are disconnected from the ER, viral particles may still be
449 assembled but only if TG molecules are present within the ER bilayer (Figure 7B). Indeed, if
450 Core binds to TGs at the ER membrane, all HCV components that are required to assemble
451 lipovirions (i.e., E1, E2, Core, TG) would be available in the same place. Therefore, proteins

452 involved in regulating TG fluxes between ER and LDs [76], such as proteins involved in LD
453 assembly or ER-LD connection should alter HCV particles' assembly. In the absence of TG
454 from the ER, the inability of Core to bind to the membrane and its subsequent degradation
455 should decrease infection (Figure 7C-D). Interestingly, DGTA1 inhibition, leading to decreased
456 lipogenesis, noticeably reduced virions [77]. Accordingly, we found that the inhibition of
457 DGATs' activity prevented Core localization to the ER bilayer (Figure 5D)

458

459 Core displaces Plin3 from the LD surface, indicating that it may perturb the LD proteome and
460 liver lipid metabolism, as corroborated by several observations. Core binding spatially
461 redistributes LDs by highjacking microtubules and dynein [78] and, thereupon, interferes with
462 lipid metabolism [79,80]. Core binding to LDs is inhibitory for the lipolytic activity of ATGL [81]
463 and, hence, increases cellular TG levels, a favorable condition for the binding of Core and
464 virion assembly (Figure 4H-I, S4H,S4J). Also, Core interferes with other proteins involved in
465 TG synthesis and packaging into LDs or degradation. An example is its interaction with DGAT1
466 [77]. It may modulate DGAT1 activity to ensure the presence of TG in the ER or directly bind
467 to DGAT1-released TGs [21]. Likewise, Rab18, an essential cellular component of LD
468 assembly, is also used by Core for targeting membranes [82]. Finally, HCV also highjacks the
469 lipophagy pathway [9,83], which, through re-esterification of fatty acids into TG in the ER
470 [31,32], may favor for Core recruitment to the ER membrane. These examples illustrate how
471 HCV may interfere with TG metabolism and packaging to grease the ER membrane for the
472 stable binding of Core and the efficient assembly of virions.

473

474 Arf1/COPI keeps the surface of LD accessible to proteins [23]; its inhibition prevents the
475 relocalization of proteins from the ER, or the cytosol, to the surface of LDs [23,57,58].
476 Accordingly, Arf1/COPI inhibition reduced Core binding to LDs (Figure 4A-E). Consequently,
477 we more often found Core in the nucleus where it is degraded (Figure 7). The occurrence of
478 this degradation may explain the decreased Core levels upon Arf1/COPI inhibition (Figure
479 S4E). Arf1/COPI also favors LD-ER physical contiguity [23,27]; its inhibition prevents the
480 exchange of proteins between the two organelles [23]. Therefore, in addition to reducing Core
481 association with LDs, Arf1/COPI inhibition may prevent the relocation of Core from the surface
482 of LDs to the ER membrane. Hence, even though the RNA levels of Core is decreased by
483 Arf1/COPI inhibition [61], the inability of the protein to efficiently access TG in LDs and the ER
484 membrane likely accounts for the severe decrease in the number of assembled virions (Figure
485 4H-I). It is interesting to quote that Arf1/COPI inhibition also prevents the association of the
486 Dengue virus capsid proteins with LDs [84]. Therefore, our findings may represent a general
487 principle by which the LD surface and ER-LD contacts serve as a folding hub for some viral
488 proteins, which may sense and use TG to promote viral assembly.

489

490 In conclusion, our results offer a crucial picture of molecular mechanisms governing the cellular
491 localization of HCV Core protein. They provide a complementary avenue for developing drugs
492 against HCV and related viruses by preventing viral proteins from modulating and accessing
493 TG in LDs or the ER membrane.

494

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499

500 **Competing interests**

501 The authors declare no competing interests.

502

503 **Author contributions**

504 The research was designed by ART, with FLC, BB, FP, AGD, and DA. DA performed
505 all cellular and in vitro experiments with the assistance of KBM, MB, and MO. BD and
506 ED generated the recombinant constructs and plasmids of the proteins. BB and FLC
507 led the virology experiments. DA and ART wrote the manuscript, reviewed by all co-
508 authors.

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746

747 **Figure 1: Core/D2 binding to the ER bilayer vs. LDs**

748 (A) Illustration of immature Core anchorage to the ER bilayer by its D3 transmembrane domain.
749 Cleavage at D3 releases mature Core. (B) HeliQuest Representation of the two amphipathic helices
750 (AHs) of D2 linked by a hydrophobic loop. (C) Co-expression of mCherry-sec61 β with Core,
751 immunolabeled by an Atto488 Antibody in Huh7 cells. Scale bar, 10 μ m. (D) (left) Expression of JFH1-
752 Core leads to Core binding to LDs, on the left. (right) Overexpressed JFH1-D1D2 localizes to the
753 nucleus. Scale bar, 10 μ m. (E) Co-expression of mCherry-sec61 β with eGFP-D2 in Huh7 cells. Right
754 panel: quantification of the signal of each protein by the LD/ER signal ratio, n=10 cells from 2
755 independent experiments. Scale bar, 10 μ m. (F) Principle for the generation of giant intracellular ER
756 vesicles (GERVs) by submitting a Cos7 cell to a hypotonic medium. (G) GERVs in Huh7 cell transfected
757 do not recruit eGFP-D2. GERVs are indicated by the luminal ER protein KDEL tagged with mRFP. Scale
758 bar, 10 μ m. (H) eGFP-Plin1 binds to GERVs in Cos7. GERVs are indicated by the luminal ER protein KDEL
759 tagged with mRFP. Scale bar, 10 μ m.

760

761 **Figure 2: D2 better folds and associates with phospholipid artificial LDs.**

762 (A) Triolein droplets and giant unilamellar vesicles (GUVs) are injected into the same observation
763 chamber and eGFP-D2 was added. D2 bound to the triolein droplets (top) and not to the GUVs
764 (bottom) of the chamber are shown. Scale bar is 40 μ m for the droplet panel, and 10 μ m for the GUV
765 panel. (B) Rhodamine-N55A-AH is bound to the droplets and bilayers of the system. (C) Quantification
766 of D2 and NS5A-AH signals on droplets and GUVs, n>3. (D) Representative FRAP analysis of eGFP-D2
767 on artificial triolein droplets, n>3, Scale bar, 20 μ m. (E) Circular dichroism (CD) spectrum of D2 in buffer
768 (green), in the presence of artificial triolein LDs covered by dioleoyl phosphatidylcholine (PC). In blue,
769 the spectrum of D2 in buffer alone. The two negative peaks at 210 and 230 nm are indicative of alpha-
770 helices. (F) D2 CD spectrum in the presence of sonicated liposomes in green. (G) Schematic illustration
771 of generated triolein droplets covered by different PC levels reported by Rhodamine-PE (Rho-PE)
772 signal. (H) D2 bound to LDs with poor in Rho-PE. n>3, scale bar is 20 μ m. (I) eGFP-D2 signal as a function
773 of the relative density of phospholipids indicated by Rho-PE signal; 10 images analyzed from triplicates.

774

775 **Figure 3: Protein crowding modulates the binding of D2 to LDs.**

776 (A) Co-expression of eGFP-D2 with mCherry-perilipins 1-3 (Plin1-3) in Huh7.5 cells. D2 displaces Plin3,
777 co-exists with Plin2, and is displaced by Plin1. Scale bar, 10 μ m. Arrowheads indicate nuclear lipid
778 droplets. (B) Quantification of the relative bound fraction of Plins and D2 on LDs, n=5, with a dozen
779 cells analyzed for each condition. (C) Schematic illustration of the binding level of D2 in the presence
780 of Plins. (D) Two examples of FRAP experiments of eGFP-D2 on LDs in Huh7.5. Insets correspond to the
781 bleached regions and are shown before, during, and after bleaching. FRAP quantifications are on the
782 right panel. Scale bar, 10 μ m. (E) Characteristic recovery rates from 23 FRAP experiments are classified
783 into three categories: inferior to 5sec, superior to 20sec, and between 5 and 20sec. The majority
784 presents a characteristic recovery rate between 5 and 20sec. (F) FRAP of eGFP-D2 on different LDs
785 within the same Huh7.5 cell. Different recovery kinetics are observed. Scale bar, 10 μ m.

786

787 **Figure 4: Arf1/COPI regulates Core localization and infection.**

788 (A) Immunofluorescence imaging of Core expressed in Huh7 in the presence or absence of Brefeldin A
789 (BFA). LDs are marked by LipidTox. Yellow arrows indicate unbound LDs, and white arrows show LD
790 with a partial core signal, n=10. Scale bar, 5 μ m. (B) Histogram quantifying the frequency of the nuclear

791 localization phenotype with or without BFA. (C) Quantification of the impact of BFA treatment on Core
792 localization per LD size classified into three categories: inferior to 1 μ m, superior to 2 μ m, and between
793 1 and 2 μ m. LDs inferior to 2 μ m are more affected in BFA conditions. (D) expression of eGFP-D2 in
794 Huh7 treated or not with BFA. Yellow arrows indicate Core-negative LDs, and the white arrows show
795 LDs partially bound by D2, or cytosolic aggregates, n=10. Scale bar, 10 μ m. (E) expression of Core in
796 Huh7 previously treated with two different siRNAs against β COP. Core is revealed by
797 immunofluorescence, n=3. Scale bar, 5 μ m. (F) Quantification of the impact of the siRNA treatment on
798 Core localization depending on LD size. (G) Schematic illustration of the complete HCV infection
799 protocol on 24h in Huh7 in the presence or absence of BFA. (H) Images of cells infected with HCV. The
800 left panel shows the Huh7 cells infected with HCV without BFA. Core is immunolabelled, visible on
801 magenta on LDs and in puncta. The right panel shows cells treated with BFA. Core's signal is drastically
802 diminished on LDs. Scale bar, 10 μ m. (I) Quantification of the intracellular and extracellular infectious
803 particles, with or without BFA treatment, in OA induction. Condition +OA and -BFA is the same as in C.
804 The minimum detection limit for infectious particles is 50 and 10 FFU / mL, respectively, for intra- and
805 extra-cellular media. The particles' formation is affected in BFA conditions.

806

807 **Figure 5: Impact of neutral lipid synthesis on the ER localization of D2.**

808 (A) A swollen Cos7 is incubated with OA and BODIPY-C12, to induce the synthesis of fluorescent
809 triacylglycerol (TG), and LipidTox. The presence of TG in the bilayer of GERVs favors the recruitment of
810 eGFP-D2: eGFP-D2, Bodipy-C12 and LipidTox signals are shown. (B) Same experiment as in (A) with
811 cells transfected with BFP-ER. (C) The recruitment of D2 to GERVs increases with the LipidTox signal in
812 OA induction cells. (D) Illustration of D2 recruitment to bilayers lacking of containing TG. (E) D2 binding
813 to GERVs is decreased by the presence of DGAT inhibitors under OA addition. (F) Quantification of the
814 recruitment level of D2 to GERVs fed with OA. (G) FRAP experiment of eGFP-D2 on a GERV, in the OA
815 loaded condition. (H) Comparison of recovery rates between LDs and GERVs. (I) GERVs are generated
816 from Cos7 cells that expressed mCherry-Sec61 and have been incubated with OA. eGFP-D2 failed to
817 efficiently bind to the GERVs. (J) The recruitment level of D2 to GERVs increased with decreased Sec61
818 signal.

819

820 **Figure 6: Triacylglycerols are determinant to D2 localization in vitro.**

821 (A) (top) Principle of the droplet-embedded vesicles (DEV) preparation before eGFP-D2 is injected into
822 the system. The Rhod-PE signal reports for phospholipids. (bottom) eGFP-D2 binds efficiently only to
823 the droplets and not to the bilayer of the DEV. n>5. Scale bar, 20 μ m. Quantification of the D2 signal
824 on the droplet vs. the bilayer region of the DEV over time is shown on the right panel. (B) Schematic
825 illustration of a DEV and its bilayer region. Water addition stretches the bilayer due to swelling,
826 delivering more TG from the droplet to the bilayer. During swelling, D2 signal in the bilayer increases.
827 The increase of the bilayer area is shown on the top right (in red). R represents the DEV radius at a
828 given time and R(0) is the initial radius. Quantification of D2 localization to the bilayer is shown on the
829 bottom right panel (in black), n=3. Scale bar, 20 μ m. (C) The swelling experiment by adding water is
830 done on a bare GUV and eGFP-D2 does not bind. The increase of the bilayer area is shown on the top
831 right. R represents the DEV radius at each time and R(0) represents the initial radius. Quantification of
832 D2 localization to the bilayer is shown on the bottom right panel, n=3. Scale bar, 20 μ m. (D) Sequence
833 alignment of Core-D2 and apoE shows a highly conserved region corresponding to the apoE reported
834 lipid-binding region. (E) Schematic illustration of an oil droplet, before and after protein addition, and
835 the associated decrease in surface tension measured by a tensiometer. ST_{in} and ST_{eq} are, respectively,

836 the initial surface tension of the bare oil/water interface and the equilibrium tension after proteins
837 adsorb to the interface. (F) Evolution of the surface tension over time in the presence of D2 for
838 triolein/water and squalene/water interfaces. (G) The relative surface tension decrease induced by D2
839 and apoE adsorption to the triolein/water vs. squalene/water interfaces are shown.

840

841 **Figure 7: Proposed model for Core cellular trafficking and conditions favorable for viral assemblies.**

842 (A) In the presence of an LD connected to the ER, Core can either fold to the LD and move back to the
843 ER membrane with TG molecules or bind directly to TG in the ER. A fraction of the protein is degraded
844 in the nucleus. (B) In the absence of LDs, Core can bind to the ER membrane, and the assembly of
845 virions can occur if TG is infused in the ER bilayer. A fraction of the protein is still degraded in the
846 nucleus. (C) In the presence of an LD physically disconnected from the ER, Core can still bind to the LD,
847 but the assembly of virions is decreased because of the absence of TG in the ER bilayer. Here, most
848 Core will proteins will end up being degraded in the nucleus. (D) In the absence of TG, Core is majorly
849 degraded, in the nucleus.

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855 **Figure S1: Characterization of Core/D2 cellular and in vitro localization**

856 (A) Expression of J6-Core leads to Core binding to LDs, the top panel. In J6-D1D2 expression in MG132-
857 treated cells, bottom panel, Core shows nuclear localization. Scale bar, 10 μm . (B) Other examples of
858 eGFP-D2 expression in Huh7 cells showing no noticeable ER membrane localization. Scale bar, 10 μm .
859 (C) Co-expression of mCherry-sec61 β with eGFP-D2 in HeLa cells reveals that D2 does not bind to the
860 ER. LDs are marked by LipidTox, n=2. Scale bar, 10 μm . (D) Expression of eGFP-D2 in HeLa cells where
861 ER is represented by a lumen ER protein in BFP. Before swelling on the top and after swelling at the
862 bottom. (E) Expression of eGFP-Plin1 in HeLa cell where ER is represented by a lumen ER protein in
863 BFP. Before swelling on the top and after swelling at the bottom. Scale bar, 5 μm .

864

865 **Figure S2:**

866 (A) Helical wheel representation of the AHs of D2 (left) and NS5A (right). Red-encircled amino acids are
867 bulky hydrophobic residues. (B) Circular dichroism (CD) spectrum of D2 in buffer (blue), in the presence
868 of artificial LDs, made with dioleoyl phosphatidylcholine (PC) at 1% (Left) or 0.1% (Right). The two
869 negative peaks at 210 and 230 nm are indicative of alpha-helices. (C) D2 CD spectrum in the presence
870 of small sonicated liposomes in 50 nm diameter range (Left) and larger liposomes formed (average
871 diameter of 500 nm of average diameter). (D) FRAP of eGFP-D2 on a triolein-in-buffer droplet shows
872 ultra-slow recovery. The arrow depicts the bleached droplet. (E) Different phenotypes of D2 in the
873 presence of LD with different PC densities, shown separately. D2 signal colocalized on droplets where
874 Rhod-PE signal is weaker. n>3, scale bar is 20 μm . (F) Another example of D2 phenotype in the presence
875 of a droplet mix of different PC densities.

876

877 **Figure S3: Characterization of D2/Core binding depending LD surface cover composition**

878 (A) Expression of mCherry-perilipins 1-3 (Plin1-3) in Huh7.5 cells. All proteins bind to LDs. (B) Schematic
879 illustration of the competition between proteins (D2 in green and Plin1 in red) at the interface of a
880 water-in-oil shrinking droplet. (C) Representative images of the fall off of eGFP-D2 from the water/oil
881 interface during shrinkage, while mCherry-Plin1 remained stably bound. The relative fluorescence
882 intensity profiles in the shown plane are embedded in the images. On right panel, the average surface
883 / volume signal during compression is plotted over the compression factor, (r_0^2 (time=0) / r^2 (at each
884 time point)). The experiment has been repeated three times. (D) Images of co-expression of Core and
885 GFP-Plin1. Scale bar, 10 μm . (E) Series of FRAP experiments of eGFP-D2 on LDs in Huh7.5. These 23
886 FRAPs are regrouped in Figure 3E. (F) Expressed pTG1-eGFP-(GGGGS)-D2 (i.e., 1 linker between eGFP
887 and D2) and pTG2-eGFP-(GGGGS)₂-D2 (i.e., 2 linkers between eGFP and D2) in Huh7.5 cells bound to
888 LDs. Scale bar, 10 μm . (G) FRAP in Huh7.5 of pTG1-eGFP-(GGGGS)-D2 and pTG2-eGFP-(GGGGS)₂-D2 on
889 LDs. Scale bar, 10 μm .

890

891 **Figure S4: Arf1/COPI impact on Core localization.**

892 (A) Additional examples of the two observed phenotypes of Core in Huh7 cells treated with BFA. White
893 and yellow arrows respectively indicate LDs having partial or no Core signal. Scale bar, 10 μm . (B)
894 Quantification of Core localization depending on LDs' size, with or without BFA. For each LD size range,
895 hundreds of LDs are quantified for the different Core localization phenotypes. (C) FRAP experiment of
896 D2 forming an arc signal around LDs in BFA condition. Scale bar, 5 μm . (D) Other examples of the
897 localization of Core in cells treated with a β COP siRNA. Scale bar, 5 μm . Quantification of the Core
898 localization depending on LD size in the control and the siRNA treatments are shown on right.
899 Hundreds of LDs are quantified from over twenty cells. (E) Immunoblotting against COPI, Core, ADRP,
900 and GAPDH in the fractions of post-nuclear supernatant (PNS) and LDs, treated or not with the β COP
901 siRNA. (F) Core LD phenotypes in the presence of β COP siRNAs. Two siRNAs and a mix of both are

902 tested. For these experiments, the Golgi is labeled with KDE-GFP. The Golgi appears more fragmented
903 in the presence of the siRNAs compared to the control cells, where KDE-GFP signal is more compact
904 and perinuclear. The Western Blot indicating the efficiency of the siRNA is shown under each treated
905 condition. Scale bar, 5 μm . (G) Schematic illustration of the complete HCV infection protocol with or
906 without induction of LDs, over 24 hours. (H) Images of Core in cells infected with the Jc1 strain, treated
907 (bottom) or not (top) with OA. Scale bar, 10 μm . (I) Images of cells infected with HCV Jc1 and
908 transfected with Plin1, 2, or 3. Plin and Core signals are shown. Scale bar, 5 μm . (J) Quantification of
909 the intracellular and extracellular infectious particles, with or without OA incubation. (K) 32 h protocol
910 diagram, with BFA washing at 24 h post-infection. (L) Quantification of the infectious particles before
911 adding BFA and after adding then washing BFA at 1 $\mu\text{g} / \text{mL}$.

912

913 **Figure S5: D2 binding to the ER**

914 (A) eGFP-D2 does not bind to the intracellular GERVs, labeled by mRFP-KDEL, in swollen Cos7. On the
915 left, control condition of Cos7 before swelling and under the co-expression of eGFP-D2 and mRFP-
916 KDEL. In the middle panel, D2 is absent from the GERV in swelling condition and cytosolic. In the right
917 panel, under swelling and OA addition, D2 localizes on intracellular GERVs. (B) Control, swelling, and
918 swelling with OA addition conditions. D2 is located to OA-rich intracellular GERVs. (C) (top) Image of
919 D2 binding to LipidTox-rich GERVs. D2 binding level to GERVs increases with the LipidTox signal. (D)
920 Protocol illustrating the use of DGAT1/2 inhibitors (iDGAT1/2) in Cos7 cells. Inhibitors are added, then
921 eGFP-D2 is transfected to cells. After 24h of transfection, live imaging is performed and the cell are
922 swollen, with replacing the cell culture medium with the hypotonic medium containing also the
923 inhibitors. The last step is OA addition. (E) In the presence of DGAT inhibitors, before swelling, D2 is
924 cytosolic; in the swollen condition, D2 is absent from GERVs. In swollen conditions, with OA addition,
925 D2 is absent from GERVs. (F) Another protocol where the DGATi are added in the meantime of OA
926 addition. (G) The same results are obtained with the precedent protocol in Figure S5D-E. (H) Other
927 FRAP experiments of D2 on GERVs. (I) The recruitment level of D2 to GERVs increases with LipidTox
928 signal (in green) and decreases with mCherry-Sec61 signal (in magenta).

929

930 **Figure S6: Comparisons between D2 and apoE.**

931 (A) Two examples of DEVs showing the binding of eGFP-D2 mostly to the droplet. (B) Rhod-NS5A-AH
932 binds to both the droplet and the bilayer region of the DEV. (C) Display of the crystalized structure of
933 apoE (PDB NCBI Reference Sequence: NP_001289617.1). (D) Sequence alignment of apoE with D1
934 Core. (E) Phylogenetic tree comparing sequence alignments of D2, Plins 1-3, apoE, apoC1, DENV NS1,
935 and Envelope glycoprotein ASFV. In contrast to perilipins, D2 is close to apoE, apoC1, and the non-
936 structural protein 1 of the Dengue virus. (F) Evolution of the surface tension over time in the presence
937 of apoE for triolein/water and squalene/water interfaces.

938

939 **Movie:** Example of a FRAP experiment carried on in a cell where two regions of LDs are simultaneously
940 bleached. eGFP-D2 showed a differential recovery rate between the LD subpopulations.

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946 **Material & methods**

947 **Plasmids and peptides.** The plasmids phCMV-core JFH1, pTG-eGFP-D2 JFH1, and mCherry-
948 NS5A that express Core, D2, and NS5A, respectively, were kindly provided by our collaborator
949 Bertrand Boson (ENS Lyon). The linkers (GGGGS)₁ or (GGGGS)₂ linkers were inserted between
950 D2 and eGFP into pTG1-eGFP-D2 and pTG2-eGFP-D2, respectively, by directed mutagenesis
951 with the following oligos: pTG1-FW 5'-AGG-GTC-TTC-CGG-ACT-CAG-ATC-TAA-C-3'; pTG1-RW
952 5'-CCG-CCA-CCC-TTG-TAC-AGC-TCG-TCC-ATG-3'; pTG2-FW 5'-GGT-GGC-GGA-GGA-TCT-TCC-
953 GGA-CTC-AGA-TCT-AAC-3'; pTG2-RW 5'-AGA-TCC-TCC-GCC-ACC-CTT-GTA-CAG-CTC-GTC-CAT-
954 G-3'. The plasmids that express PLIN peGFP-Plin 1-3, were kindly provided by the team of
955 University of Cambridge Metabolic Research Laboratories. The human protein ApoE (Sigma-
956 Aldrich). Peptide J6-D2 was obtained from François Penin and has the following sequence
957 NLGKVIDTLTCGFADLMGYIPLVGAPLGGAAARALAHGVRVLEDGVNYATGN. For comparison, the
958 JFH1-D2 sequence is: NVGKVIDTLTCGFADLMGYIPVVGAPLSGAARAVAHGVRVLEDGVNYATGN.
959 To mark Golgi, we used the plasmid KDE-GFP (Dipeptidyl peptidase IV in which the
960 extracellular domain had been replaced by the GFP sequence to restrict protein localization
961 to the Golgi apparatus [85]), was kindly given by Professor Christian Poüs (Paris-Sud
962 University, France).

963
964 **Expression constructs.** pFK-JFH1/J6/C-846_dg plasmid was kind gift from R. Bartenschlager
965 [86]. phCMV-core JFH1 and phCMV-core J6 plasmids were previously described [46]. phCMV-
966 core FP- JFH1 and phCMV-core CS- J6 were constructed by inserting a stop codon by
967 mutagenesis after the amino acid 171 of the core protein in phCMV-core JFH1 and phCMV-
968 core J6, respectively.

969
970 **Production of the recombinant peptide eGFP-D2.**

971 The eGFP-D2 DNA sequence was cloned into pET15b (Novagen, Merck) between NdeI/BamHI
972 restriction sites. Induction of eGFP-D2 expression in BL21pLys (Invitrogen, Thermofischer) was
973 done at +37°C for 3h, after addition of 0.5 mM IPTG (Sigma-Aldrich) at 0.5DO of growth in LB
974 supplemented with 100µg/mL ampicillin. Bacterial were centrifuged at 5000g for 15min at
975 4°C. The pellet was resuspended in 35mL of 20mM phosphate buffer at pH = 7.4,
976 supplemented with 500mM NaCl, 10% glycerol, 1mM PMSF and 50µg/mL lysozyme (Sigma-
977 Aldrich, Merck) and submitted to French Press extraction at 1500 PSI (Thermofischer). After
978 centrifugation of the crude lysate at 25000g, 4°C for 30min, the supernatant was filtered on
979 0.45µm, and 0.20µm cellulose membranes (Merck) before loading on 1mL HisTrap HP affinity
980 column (Amersham, GE Healthcare) conditioned with extraction buffer. Protein eGFP-D2 was
981 eluted with 250mM imidazole (Sigma-Aldrich) after linear gradient from 40mM to 1M
982 imidazole in 15CV (Akta purifier Amersham, GE Healthcare). After analysis of 20µL of each
983 fraction on Novex BisTris, 4-12% PAGE (Life Technologies Thermofischer) revealed with
984 Coomassie blue (PageBlue, Thermofischer), fractions containing 33kDa proteins were pooled
985 and dialyzed O/N at +4°C in 20mM phosphate buffer pH = 7.4 supplemented with 50mM NaCl,
986 10% glycerol. The dialyzed fractions were loaded on a MonoQ equilibrated with dialysis buffer
987 (Amersham, GE Healthcare) and submitted to a linear gradient from 50mM to 1M NaCl for
988 anionic exchange. The protein eGFP-D2 was eluted with 250mM NaCl, analyzed on PAGE, and
989 quantified by spectrophotometry by measuring absorbance in UV (from 190nm to 340nm) and
990 at 488nm. We obtained 1,5 mg of highly purified eGFP-D2 protein at 23 µM.

991

992 **Antibody.** Monoclonal antibodies (references 19D9D6, Bio-Merieux) of mice directed against
993 Core HCV proteins (recognize the Core D1 domain) were used to detect the location of Core
994 in the transfected cells. The secondary antibodies of donkey anti-mouse marked with the
995 Alexa 555 anti Monoclonal antibodies 19D9D6 (provided by our employees of the ENS, Lyon,
996 references A-31570, Invitrogen). These antibodies are used in immunofluorescence, diluted
997 to 1/1000. For virology experiments: Mouse anti-core 19D9D6 (kind gift from C. Jolivet,
998 bioMérieux, Lyon, France), Mowiol 40-88 (Fluka, Switzerland), Oleic Acid-Albumin and BFA
999 (Sigma Aldrich, France), BODIPY 493/503, LipidTox Deep Red, Hoechst 33342 (Molecular
1000 Probes Europe BV, The Netherlands) and Gene Jammer (Agilent, United States) were used
1001 according to the manufacturer's instructions.

1002
1003 **Cell Culture and reagents.** Huh7, Huh7.5, and HeLa cells were maintained in Dulbecco's
1004 modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine
1005 serum (Life Technologies), 4.5 gL⁻¹ D-glucose, 0.1 gL⁻¹ sodium pyruvate (Life Technologies), and
1006 1% penicillin-streptomycin (Life Technologies). The cells were cultured at 37°C under a 5%CO₂
1007 atmosphere. HeLa (human cervical carcinoma obtained from the American Type Culture
1008 Collection) cells were maintained in DMEM supplemented with 10% (vol/vol) FBS, 2 mM L-
1009 glutamine, penicillin–streptomycin, 1% sodium pyruvate, and 1% nonessential amino acids.
1010 For virology experiments: Huh7.5 cells (king gift from C. Rice) were grown in Dulbecco's
1011 modified minimal essential medium (DMEM, Invitrogen, France) supplemented with 100U/ml
1012 of penicillin, 100µg/ml of streptomycin, and 10% fetal bovine serum.

1013
1014 **Transfection and co-transfection.** When indicated, Huh7 or HeLa cells (60–70% confluence)
1015 plated into a 35 mm cell-culture MatTek dishes (with a glass coverslip at the bottom), (MatTek
1016 Corp. Ashland, MA). were exposed for 1 h to 350 µM oleic acid (OA) coupled to bovine serum
1017 albumin (BSA) (1% vol/vol) to induce LD formation, and then cells were transfected with 3 µg
1018 of plasmid DNA/ml using Polyethylenimine HCl MAX (Polysciences) following the
1019 manufacturer's instructions. For coexpression competition experiments, mCherry- or GFP-
1020 tagged plasmid constructs in equal concentrations (1.5–2 µg for each one) were transfected
1021 into cells. Cells were imaged at 24 h after transfection. For virology experiments: HCV core or
1022 GFP-PLIN expression. HCV core or eGFP-PLIN expression plasmids were transiently expressed
1023 in Huh7.5 cells by transfection with GeneJammer transfection reagent according to the
1024 manufacturer's instructions. Briefly, 2.10e⁵ Huh7.5 cells seeded in 6-well plates were
1025 transfected with 2µg of DNA and 4µL of GeneJammer diluted in Optimem medium for 8h or
1026 16h. The medium was then replaced by a fresh medium for HCV core expression studies or by
1027 a medium containing Jc1 at MOI=0.2 for eGFP-PLIN studies, and cells were then cultured for
1028 the indicated time.

1029
1030 **HCVcc production and titration.** HCVcc production procedures were described previously
1031 [86]. Supernatants infectivity titers were determined as focus-forming units (FFU/ml). Serial
1032 dilutions of supernatants were used to infect Huh7.5 cells, FFUs were determined 3 days post-
1033 infection by counting NS5A-immunostained foci. For determining intracellular infectivity,
1034 infected cells were washed with PBS and harvested after trypsin treatment and centrifuged
1035 for 4 min at 400xg. Cell pellets were suspended in medium and subjected to 4 cycles of freeze
1036 and thaw with liquid nitrogen. Serial dilutions of this cell lysate were then used for
1037 supernatants to determine the intracellular infectivity.

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FRAP experiments. For FRAP experiments, we bleached the signal on a cluster of drops and monitored the increase of signal during recovery. The background signal, for example, from the cytosol, was removed from the recorded signal, which was at the end normalized by intrinsic bleaching of non-bleached areas. We next used GraphPad Prism to fit the FRAP recovery curves with a nonlinear regression and the exponential one-phase association model. The characteristic recovery time that corresponds to the time it takes for fluorescence intensity to reach half the maximum of the plateau level is obtained by the software.

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Cellular Fractionation. For purification of LDs from cells expressing fluorescently tagged LD proteins, cells from five 150 cm dishes were harvested, washed once in ice-cold PBS, and lysed using a 30G needle in 1 ml Tris-EDTA (20-10 mM, respectively) buffer containing complete protease and phosphatase inhibitor tablets (ThermoFisher) at pH 7.5. For LD isolation, 1 ml of cell lysates was mixed with 1 ml of 60% sucrose in Tris-EDTA buffer supplemented with protease inhibitors, overlaid with 20, 10, and 0% buffered sucrose on top of one another in 5 ml Ultra-Clear centrifuge tubes (Beckman). Gradients were centrifuged for 16 h at 100,000 × *g* and 4°C, using an SW60 rotor in a Beckman L8-70 centrifuge, and 300 µl was collected from the top as the LD fraction.

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Western Blot. The different purified cell fractions were migrated on a 10% SDS PAGE gel (cast in the laboratory), and the bands of the gel were electro-transferred onto a nitrocellulose membrane (Schleicher & Schuell). The membrane was then incubated with an anti-Core (MA1-080, ThermoFisher)/ ADRP (ab52355, Abcam) / COPI (ab2899, Abcam) / actin monoclonal antibody (sc-47778 HRP, SantaCruz), from Abcam, followed by incubation with HRP-conjugated secondary IgG and visualized by a Pierce ECL kit (Thermofisher).

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Immuno-fluorescence (IF) and confocal microscopy imaging. Huh7 cells grown on 35 mm cell-culture MatTek dishes were transfected with Core plasmid. 24 h post-transfection, Cells were fixed with 4% formaldehyde for 15 min; this was followed by three washes in phosphate-buffered saline (PBS), then permeabilized with 0.3% Triton X-100 for 10min at room temperature. Fixed cells were then incubated for the night with anti-Core antibody diluted 1/1000 in 3% BSA/PBS, washed and stained for 1h with fluorescent secondary antibody (anti-Mouse AlexaFluor-555, Molecular Probes Europe BV, The Netherlands) in 3% BSA/PBS. LDs were stained with 10µg/mL BODIPY 493/503 or with LipidTox Deep Red according to the manufacturer's instructions. Cells were washed and mounted on microscope slides with ProLong Gold Antifade Mountant with 4',6-diamidino-2-phenylindole (Thermo Fisher Scientific), and the fluorescently tagged protein localization was determined using a Leica TCS SP8 confocal microscope with a 63× immersion oil objective (1.3 NA).

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GFP fluorescence was excited at 488 nm, and emission was detected between 490 and 550 nm, while M-cherry-tagged protein fluorescence was excited at 588, and emission was detected between 600 and 650 nm. LDs were detected with either BODIPY 493/502 (excitation: 502 nm; emission: 515–560 nm) or LipidTox Deep Red (excitation: 558 nm; emission: 590–700 nm).

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For virology experiment: Huh7.5 cells grown on uncoated 14mm-diameter glass coverslips were infected with HCVcc at MOI of 0.2. When stated, the viruses were removed 8h post-infection and replaced by media containing 1µg/mL of BFA or diluent (EtOH) for 24h. At indicated times post-infection, cells were washed with PBS, fixed with 3% paraformaldehyde

1086 in PBS for 15min, quenched with 50mM NH₄Cl, and permeabilized with 0.1% Triton X-100 for
1087 7min. Fixed cells were then incubated for 1h with anti-core antibody diluted 1/1000 in 1%
1088 BSA/PBS, washed and stained for 1h with fluorescent secondary antibody (anti-Mouse
1089 AlexaFluor-555, Molecular Probes Europe BV, The Netherlands) in 1% BSA/PBS. LDs were
1090 stained with 10μg/mL BODIPY 493/503 or with LipidTox Deep Red 1/200 according to the
1091 manufacturer's instructions. Cells were washed three times with PBS, stained for nuclei with
1092 Hoechst (Molecular Probes) for 5min when stated, washed, and mounted in Mowiol prior to
1093 image acquisition with LSM-710 confocal microscopes.

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1095 **Interference RNA and inhibitor treatment.** For COPI inhibition, Huh7 cells were grown on 35
1096 mm cell-culture MatTek dishes and were treated with siRNA (Dharmacon) complexed with
1097 HiPerFect Transfection Reagent (Qiagen), according to the manufacturer's instructions for
1098 24h. For siRNA experiment, two lots are used, Lot1 (Horizon, D-017940-04-0002), Lot2
1099 Horizon, D-017940-03-0002). Then treated cells were incubated with OA and transfected with
1100 Core plasmid. 24 h post-transfection, cells were fixed, and IF protocol was applied. For
1101 Brefeldin A inhibition, it is added 4h post-transfection, and cells are imaged 20h after
1102 inhibition. For the DGAT1 (Sigma PF-04620110) and DGAT2 (Sigma PF-06424439) inhibitors
1103 used in Cos7, the dilution applied was 1/1000 for a final concentration of 3μg/mL. In the GERV
1104 protocol (Figure 5D, S5D-E), the inhibitors were added when the cell medium is replaced by a
1105 hypotonic medium, and/or just before cell transfection (Figure S5F-G).

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1107 **Circular Dichroism CD.** The CD spectra were obtained on a Jobin-Yvon CD6 device. The scan
1108 speed is set at 0.2 nm / s. The measurements were carried out in a quartz tank 1 mm in
1109 diameter. A blank (buffer solution) is made before each measurement and is subtracted from
1110 the protein spectra. For each measurement, three spectra were recorded to increase the
1111 signal-to-noise ratio. The final concentrations of peptides used are between 90 and 150 μM
1112 in 10 mM sodium phosphate buffer (pH 7.4) supplemented with 100 mM potassium chloride
1113 KCl and 2 mM MgCl₂ (buffer called PKM). The results are presented in standardized Δε values
1114 on the basis of the mass of an amino acid residue of 110 KDa using the formula: $\Delta\epsilon = \Delta A / (l * C * n)$,
1115 with Δε which is the difference of molar extinction coefficient in M⁻¹.cm⁻¹, ΔA is the
1116 absorption difference, l is the optical path in cm, C is the molar concentration in mol / L and n
1117 is the number of residues of the analyzed peptide. The sensitivity of the dichrograph is δ (ΔA)
1118 = 10⁻⁶.

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1120 **Bioinformatics.**

1121 **Heliquest** was used for α-helix projections (www.heliquest.ipmc.cnrs.fr). Hydrophobic AAs are
1122 illustrated in yellow. For D2, we use the two amino-acids sequences NVGKVIDLTLCGFADLMGY and
1123 ARAVAHGVRVLEDGVNYATGN, which represent the two AHs of D2, respectively.

1124 For data presented in Figure 6D and Figure S6D, EMBOSS Water pairwise sequence **local alignment**
1125 **tool**, https://www.ebi.ac.uk/Tools/psa/emboss_water/, was used to study the similarity between
1126 human APOE, APOC1, PLIN1-3, HCV JFH1 core, D1 and D2, Flavivirus Envelope glycoprotein (from
1127 African swine fever virus (ASFV) and Nonstructural protein NS1 (from Dengue virus (DENV)).

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1130 **Shrinking experiments.** *In vitro* experiments were performed in HKM buffer: 50 mM HEPES,
1131 120 mM potassium acetate, and 1 mM MgCl₂ (in Milli-Q water) at pH 7.4. To create a buffer
1132 in oil drops, 4 μl of a buffer-diluted LD fraction was mixed with 40 μl of triolein vortexing.

1133 About 100 drops were formed and imaged in the field of observation. For shrinking
1134 experiments, aqueous drops bounded by the proteins were imaged for 10–30 min on glass
1135 plates during water evaporation.

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1137 **Giant Unilamellar Vesicles and Artificial Lipid Droplets Formation GUVs.** GUVs were
1138 prepared by electroformation. Phospholipids and mixtures thereof in chloroform at 0.5 mM
1139 were dried on an indium tin oxide (ITO)-coated glass plate. The lipid film was desiccated for 1
1140 h. The chamber was sealed with another ITO- coated glass plate. The lipids were then
1141 rehydrated with a sucrose solution (275 mOsm). Electroformation is done using 100 Hz AC
1142 voltage at 1.0 to 1.4 Vpp and maintained for at least 1 h. This low voltage was used to avoid
1143 hydrolysis of water and dissolution of titanium ions glass plate. GUVs were either stored in
1144 the chamber at 4°C overnight or directly collected with a Pasteur pipette.

1145 To prepare the artificial lipid droplets (aLDs), 5 mL of the lipid oil solution was added to 45 mL
1146 of HKM buffer. The mixture was sonicated. The diameter of the resulting droplets is a few
1147 hundred nanometers.

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1149 **Droplet-embedded vesicles' preparation:** Unless mentioned, *in vitro* experiments were
1150 performed in the following HKM buffer: 50 mM Hepes, 120 mM Kacetate, and 1 mM MgCl₂
1151 (in Milli-Q water) at pH 7.4 and 275±15 mOsm. All **GUVs** were 70 % DOPC 29% Rhodamine-
1152 DOPE 1% (w/w) Rhodamine-DOPE. GUVs were prepared by electro-formation. Phospholipids
1153 and mixtures thereof in chloroform at 0.5 mM were dried on an indium tin oxide (ITO) coated
1154 glass plate. The lipid film was desiccated for 1 h. The chamber was sealed with another ITO
1155 coated glass plate. The lipids were then rehydrated with a sucrose solution (275±15 mOsm).
1156 Electro-formation is performed using 100 Hz AC voltage at 1.0 to 1.4 Vpp and maintained for
1157 at least 1 h. This low voltage was used to avoid hydrolysis of water and dissolution of the
1158 titanium ions on the glass plate. GUVs were either stored in the chamber at 4°C overnight or
1159 directly collected with a Pasteur pipette. To prepare the aLDs, 5 µL of the oil was added to 45
1160 µL of HKM buffer. The mixture was sonicated. The diameter of the resulting droplets is on the
1161 order of a few hundred nanometers. To make DEV, GUVs were then incubated with the aLDs
1162 for 5 min. The GUV-LD mixture was then placed on a glass coverslip pretreated with 10%(w/w)
1163 BSA and washed three times with buffer.

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1165 **Giant ER vesicles' generation.** For cell swelling experiments, Cos7 cells were first transfected
1166 for 24 h with the eGFP-D2 plasmid. The culture media was next replaced by a hypotonic
1167 culture media (DMEM: H₂O, 1:20). The cells were then incubated at 37°C, 5% CO₂ for 5
1168 minutes, to induce ER-vesicles. For analyzing D2 localization, cells were imaged directly after
1169 swelling. The cells were next incubated with 400 µM OA conjugated to 1% (w/w) BSA in
1170 DMEM: H₂O (1:20) media at 37°C to induce triacylglycerol (TG) synthesis. Then BODIPY-C12
1171 558/568 was also added to OA (1:1000) to induce and visualize fluorescent TG localization. Z-
1172 stacks imaging of entire cells was done before and 15 min after OA administration. Z-stacks of
1173 the entire cell were imaged after swelling, before feeding, and 15 minutes after feeding.
1174 mCherry-KDEL and ER-BFP or mCherry-Sec61β are used as a marker of ER lumen or ER
1175 membrane, respectively.

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1177 **GUV Swelling experiments.** MilliQ water was added to the bulk HKM buffer of the GUVs: 10
1178 µL of MilliQ was added to the 100 µL of HKM buffer.

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1180 **Interfacial tension measurements (γ)**

1181 We used an oil-drop tensiometer (Mitsche et al., 2010; Mitsche and Small, 2013; Meyers et
1182 al., 2013) designed by Teclis Instruments (Tassin, France) to measure the interfacial tension
1183 (γ) of lipid–water interfaces. All experiments were conducted at $25.0 \pm 0.2^\circ\text{C}$ in a thermostated
1184 system and repeated at least twice.

1185 For the creation of TO–Water or squalene-Water interfaces (Mitsche and Small, 2011), TO or
1186 squalene Sq drops ($5 \mu\text{L}$) were formed at the tip of a J-needle submerged in 5 ml of bulk buffer.
1187 Their surface area was $\sim 30 \text{ mm}^2$ (diameter = 3.1 mm). The buffer was 5 mM HKM (50 mM
1188 Hepes, 120 mM K acetate, and 1 mM MgCl_2 (in Milli-Q water)
1189 at pH 7.4. The TO–W interface stabilized at $\gamma_{\text{TO}} = 32.0 \pm 1 \text{ mN/m}$. The Sq–W interface
1190 stabilized at $\gamma_{\text{Sq}} = 22.0 \pm 1 \text{ mN/m}$.

1191 Adsorption of proteins (i.e., D2, ApoE) to the interface decreases γ to a nearly constant value
1192 defined as equilibrium tension (γ_{eq}). D2 or ApoE peptide was added to the bulk phase to obtain
1193 final protein concentrations of $0.18 \mu\text{M}$ and $0.014 \mu\text{M}$, respectively. As peptide adsorbed to
1194 TO–W and Sq–W interfaces, γ was monitored until it fell to an equilibrium value (γ_{eq}).

1195 Relative tension decrease was defined as the difference in γ between a pure TO–W or Sq–W
1196 interface ($\gamma_{\text{TO}} = 32.0 \text{ mN/M}$; $\gamma_{\text{SQ}} = 22.0 \text{ mN/M}$) and the interface at the equilibrium with
1197 bound peptide (Relative tension decrease = $(\gamma_{\text{TO}} - \gamma_{\text{eq}})/\gamma_{\text{TO}}$ and (Relative tension decrease =
1198 $(\gamma_{\text{SQ}} - \gamma_{\text{eq}})/\gamma_{\text{SQ}}$).

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1200 **Quantification and statistical analysis**

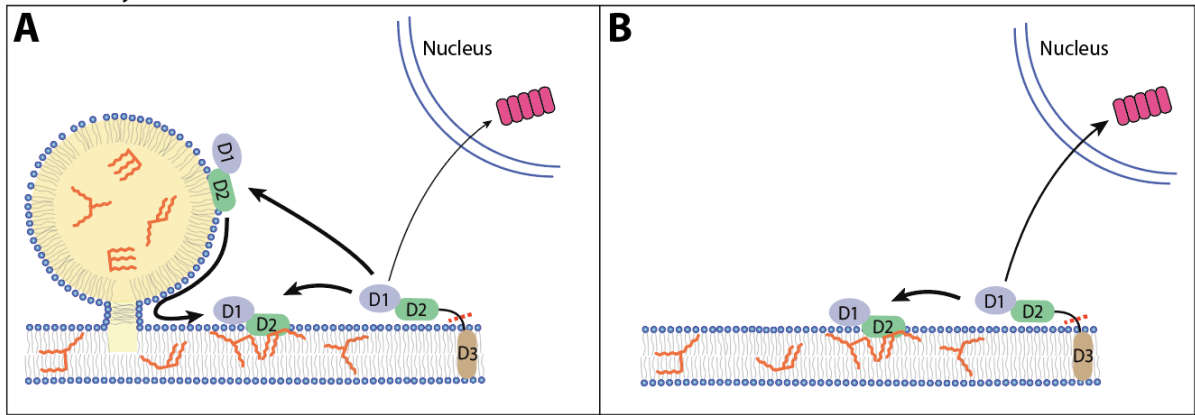
1201 Fluorescent signal quantification at LD or GUV surface

1202 To quantify the recruitment of the fluorescent protein at the surface of LDs, we used the radial
1203 angle profile plugin of ImageJ software. This plugin measures the average signal intensity
1204 along the perimeter of concentric circles. It results in a plot of the intensity profile of a circular
1205 object for various positions relative to its center. We chose the maximum intensity profile as
1206 a measurement of recruited peptide density. The same method was used to quantify the
1207 phospholipid density covering droplets. For the competition experiment, protein
1208 concentrations on LDs were derived from the mean fluorescence measured on LDs in each
1209 channel (Plins vs. D2, Figure 3); each experimental point corresponds to the average of the
1210 signal on 10–20 LDs. Values from 15–20 cells were combined, and the SD was calculated for
1211 statistical analysis. Values from 15–20 cells were combined, and the SD was calculated for
1212 statistical analysis.

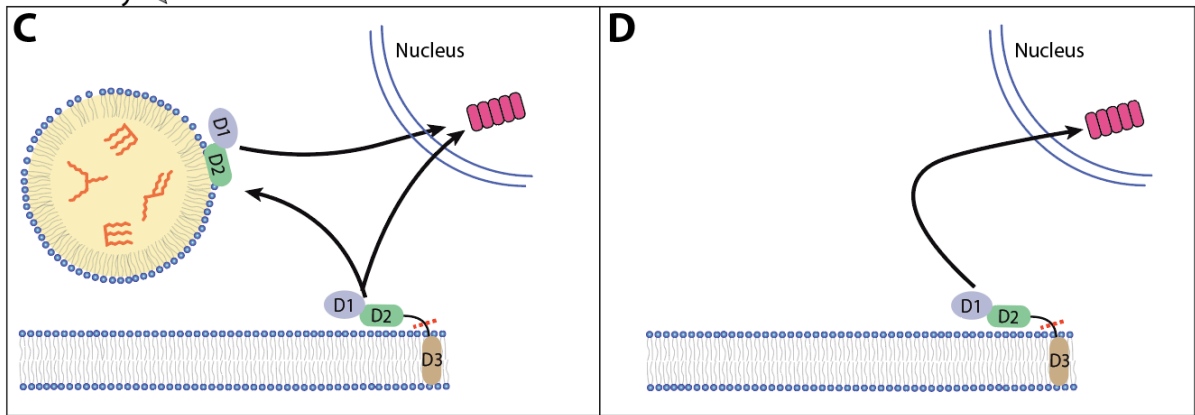
1213

Infectivity ↗

Triglyceride Proteasomal degradation

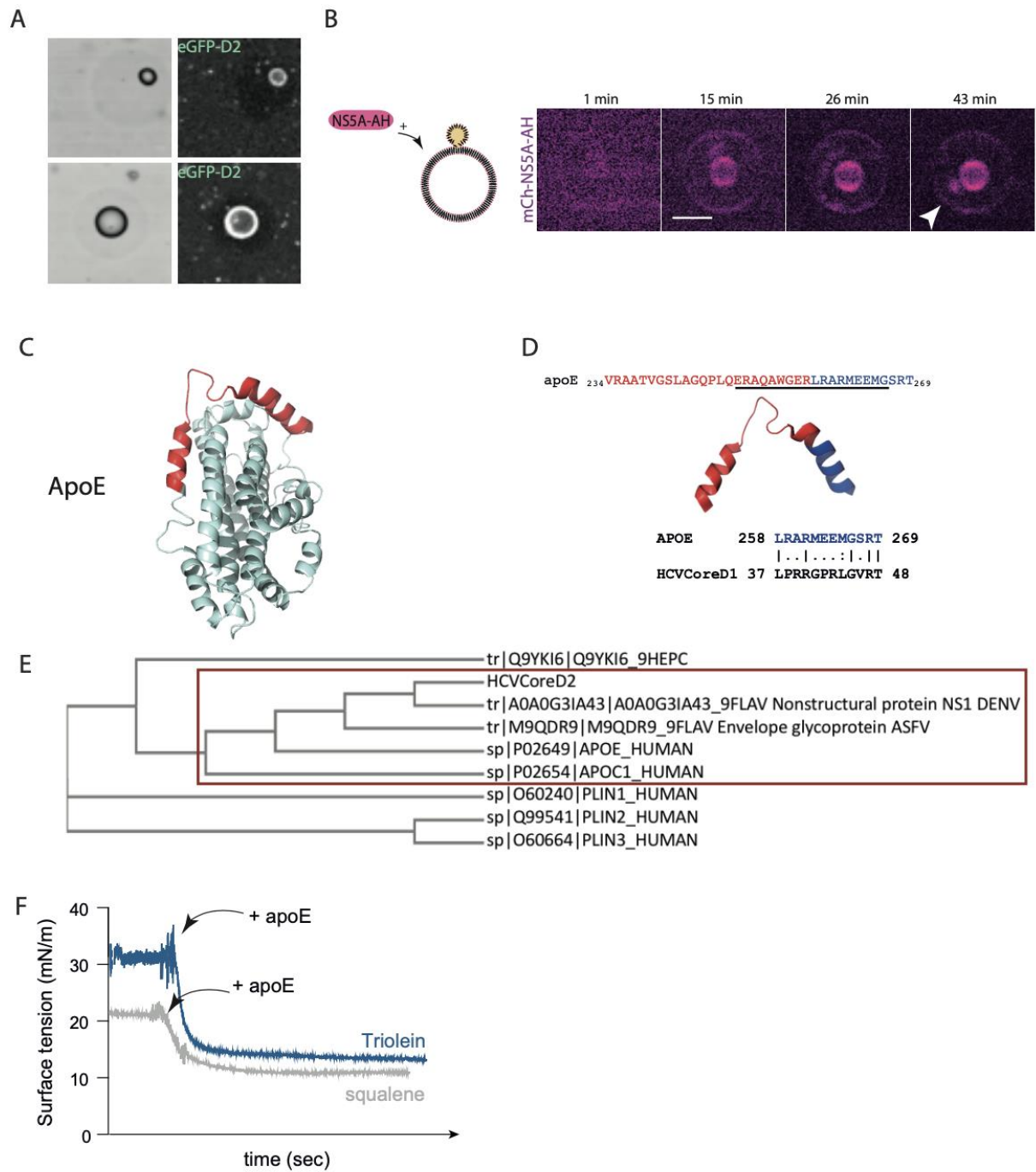


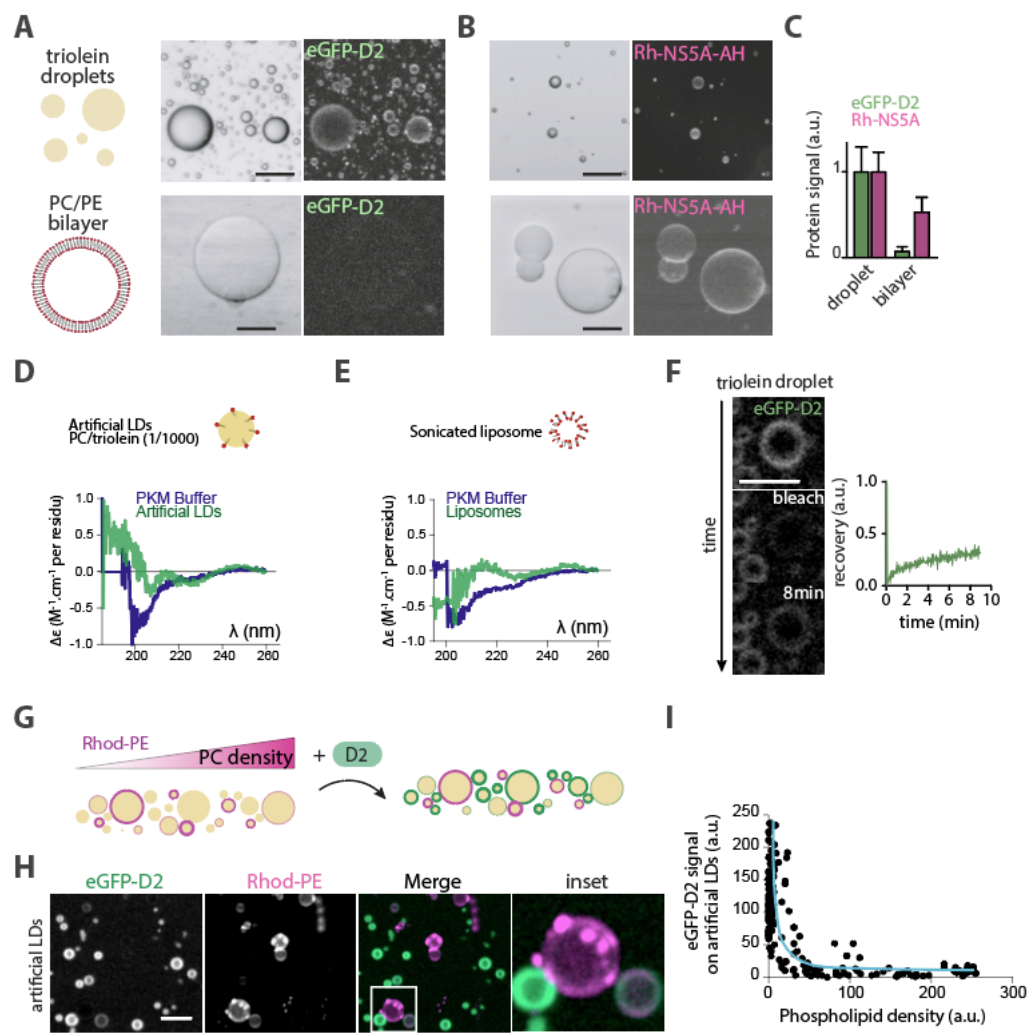
Infectivity ↘



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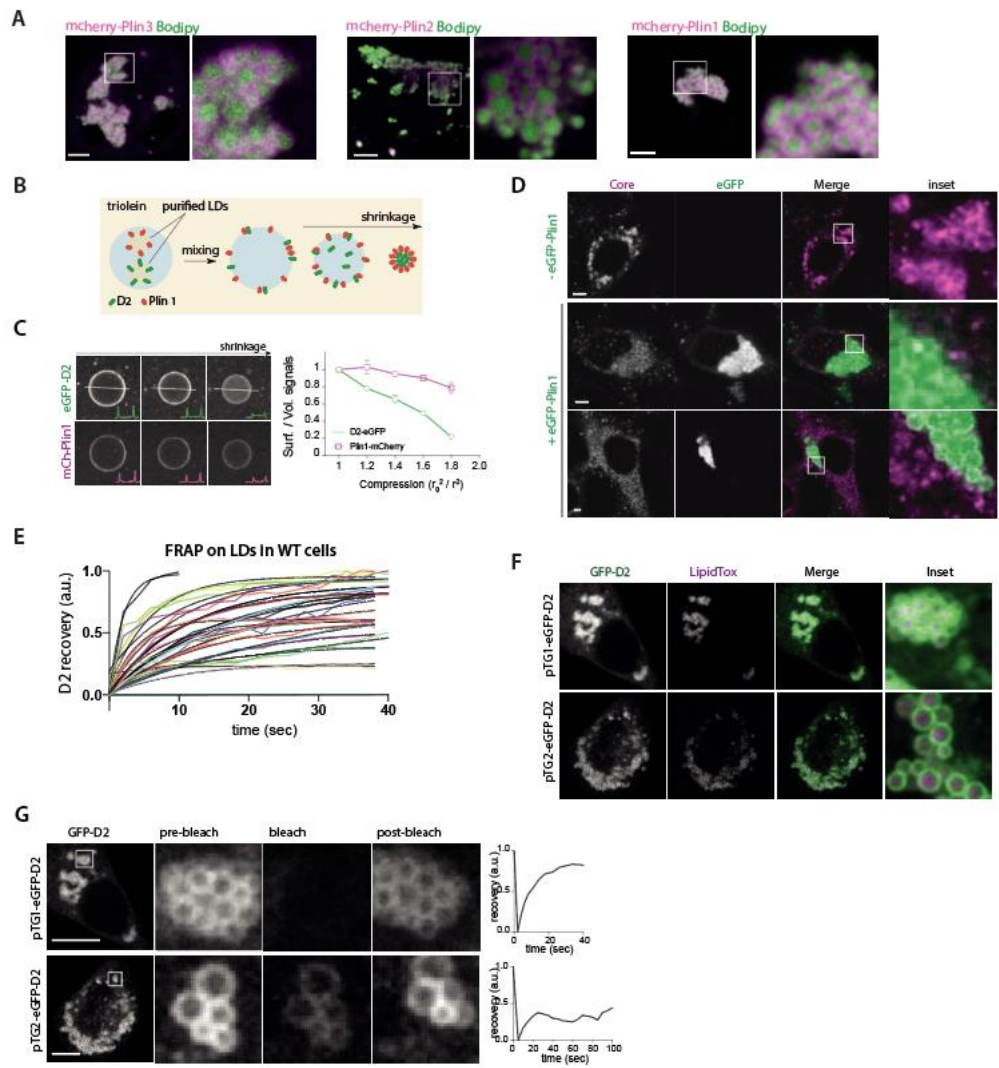
Figure 7





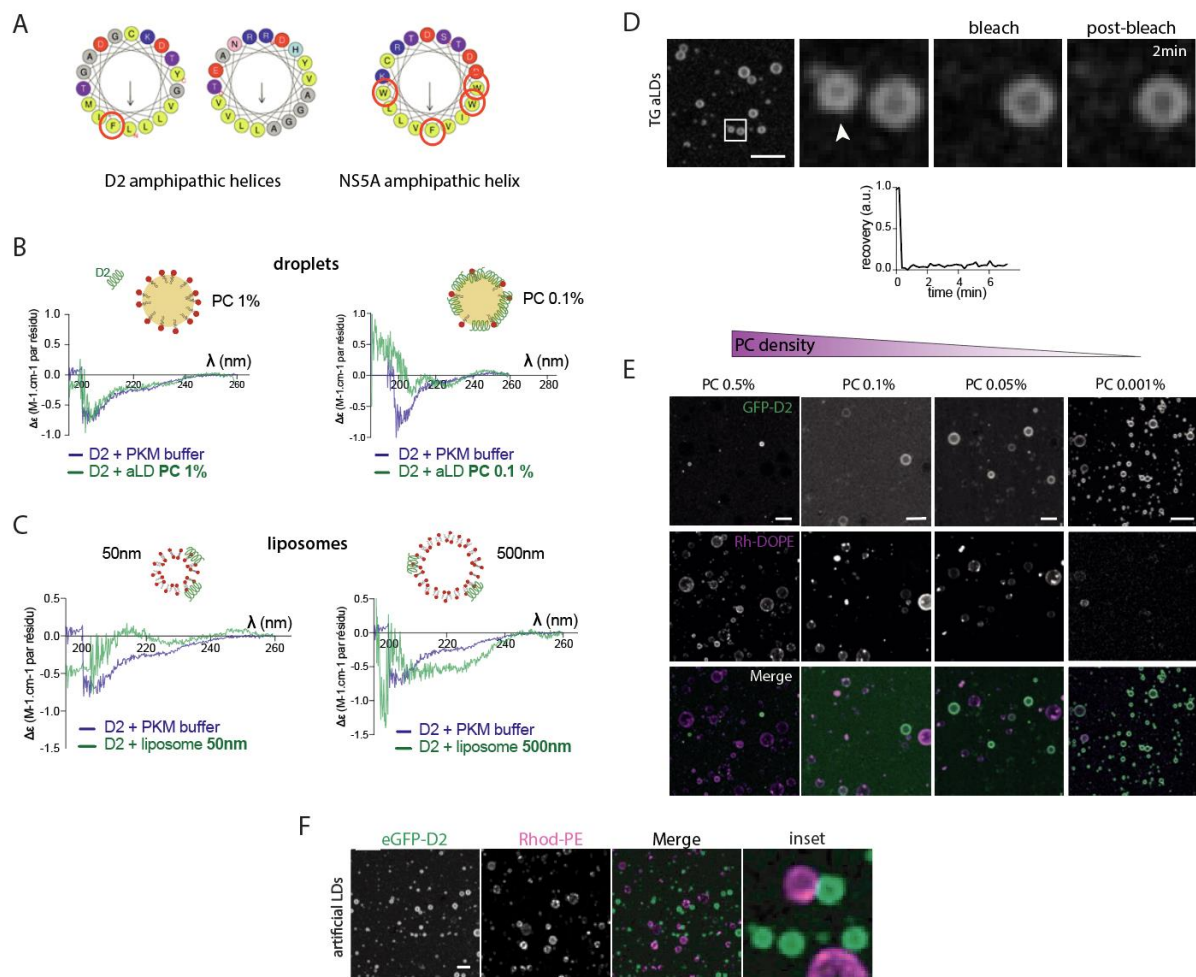
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Figure 2



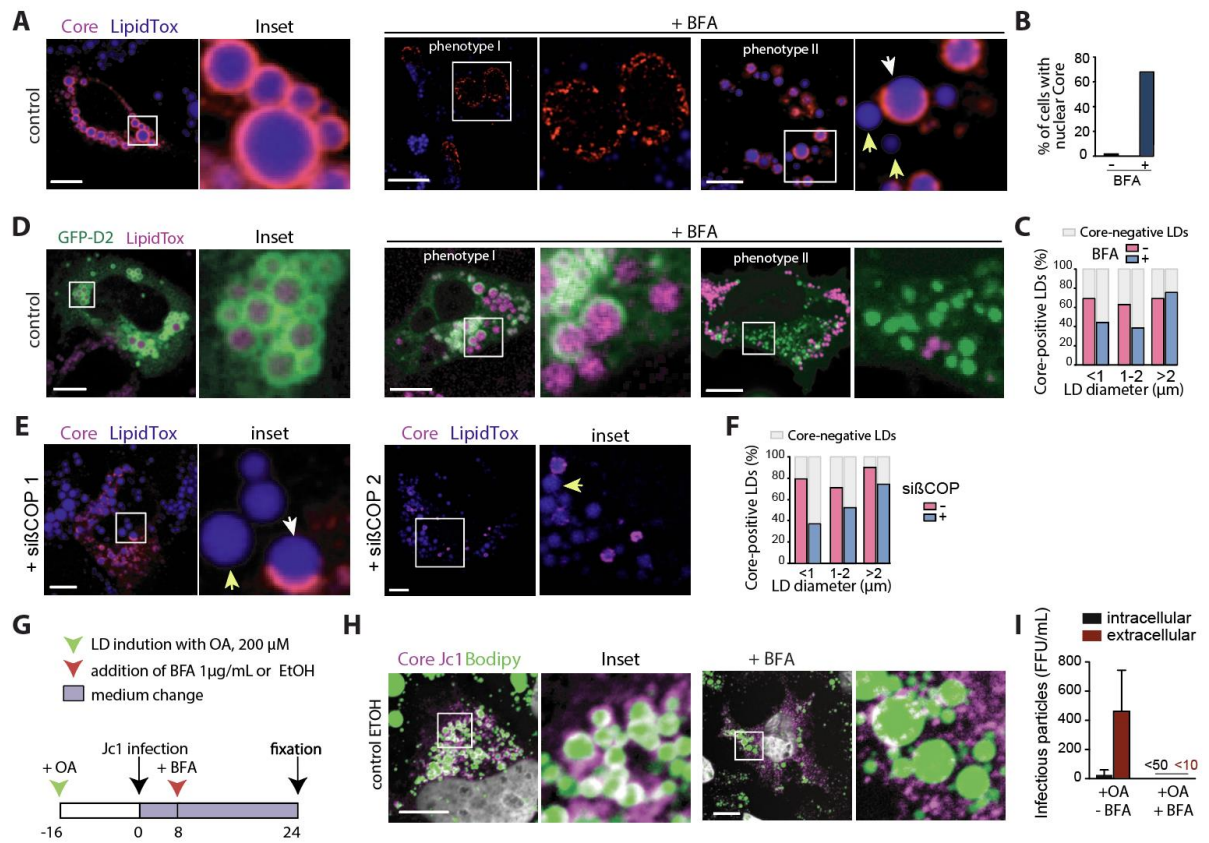
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Figure S3



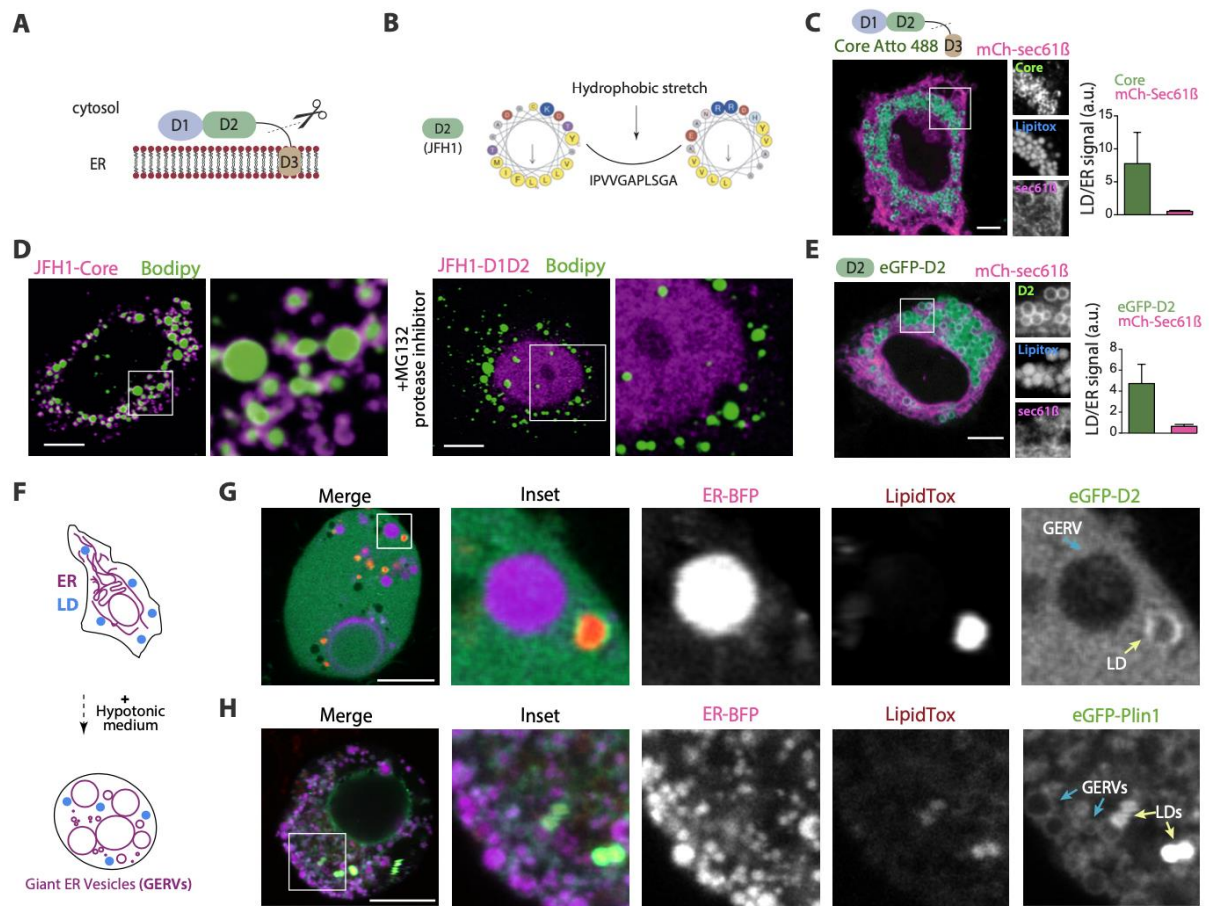
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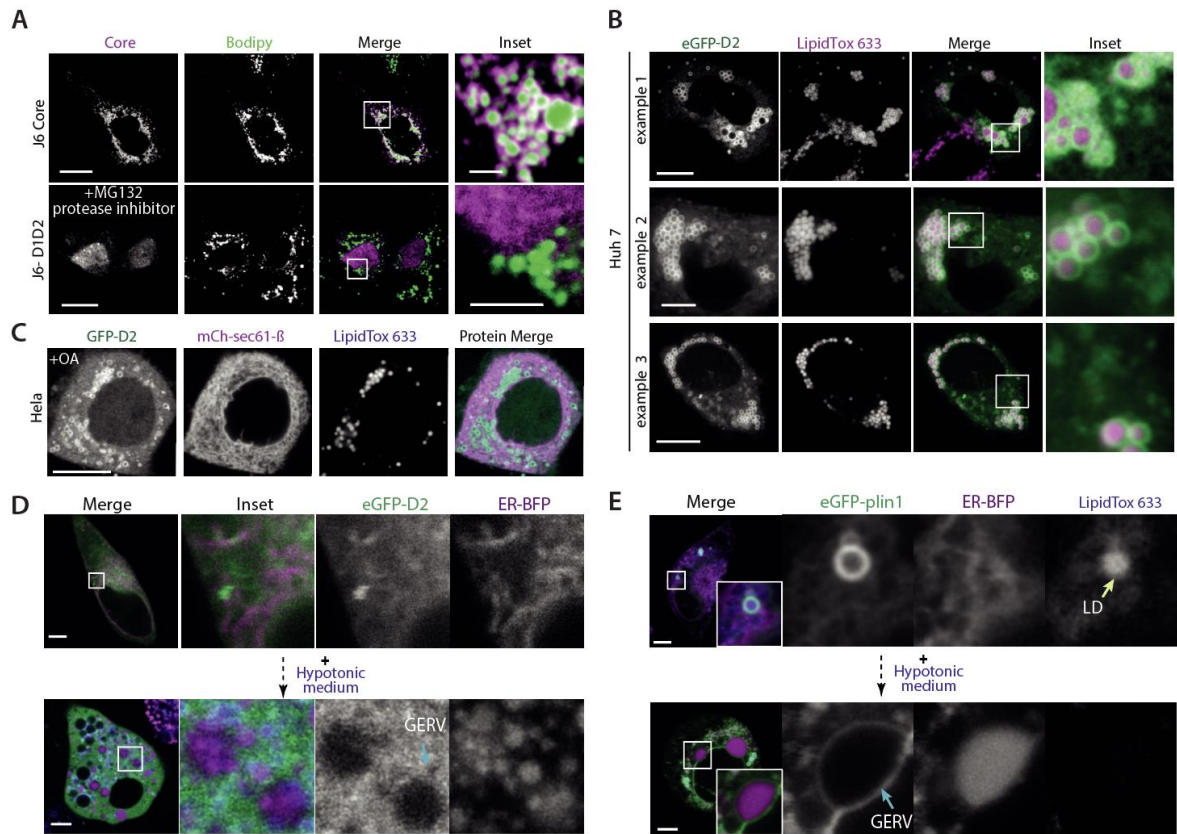
Figure S2



1220

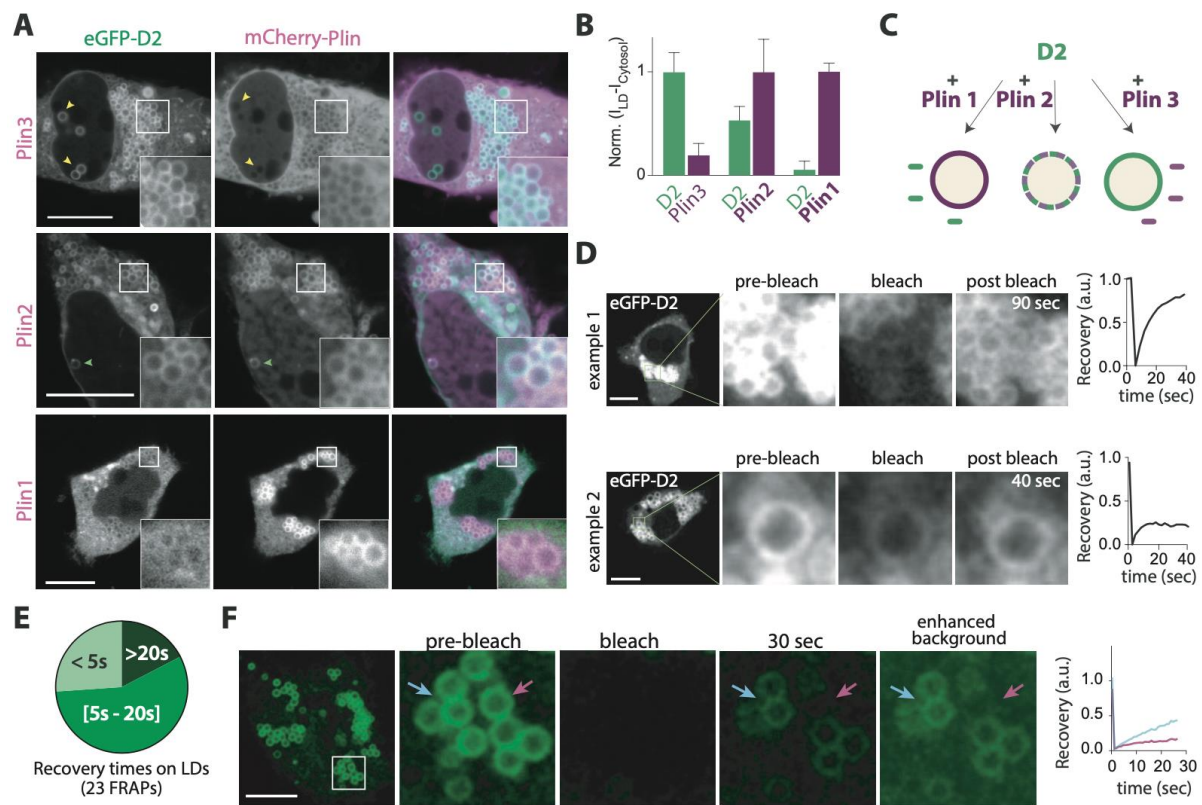
Figure 4





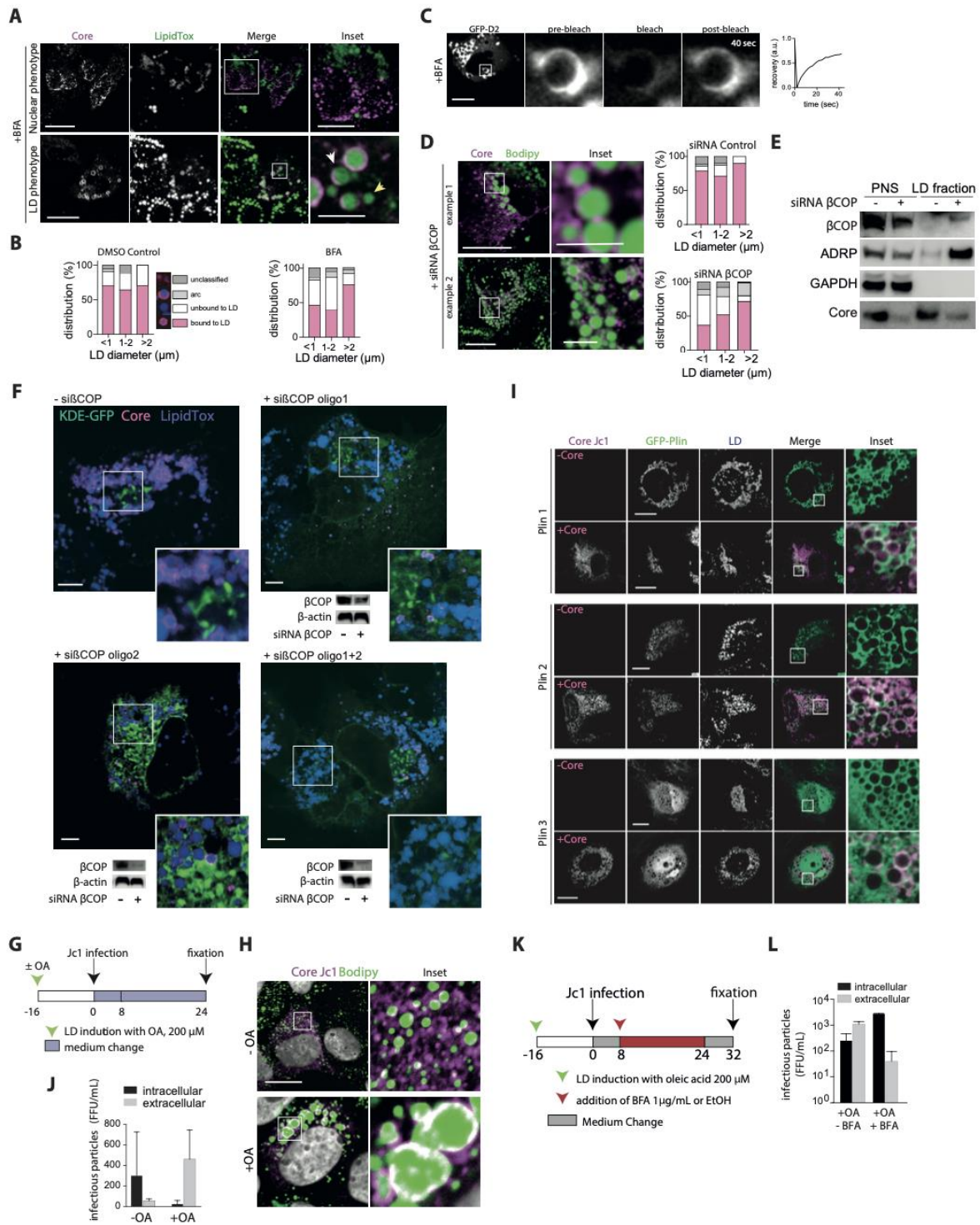
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Figure S1



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Figure 3



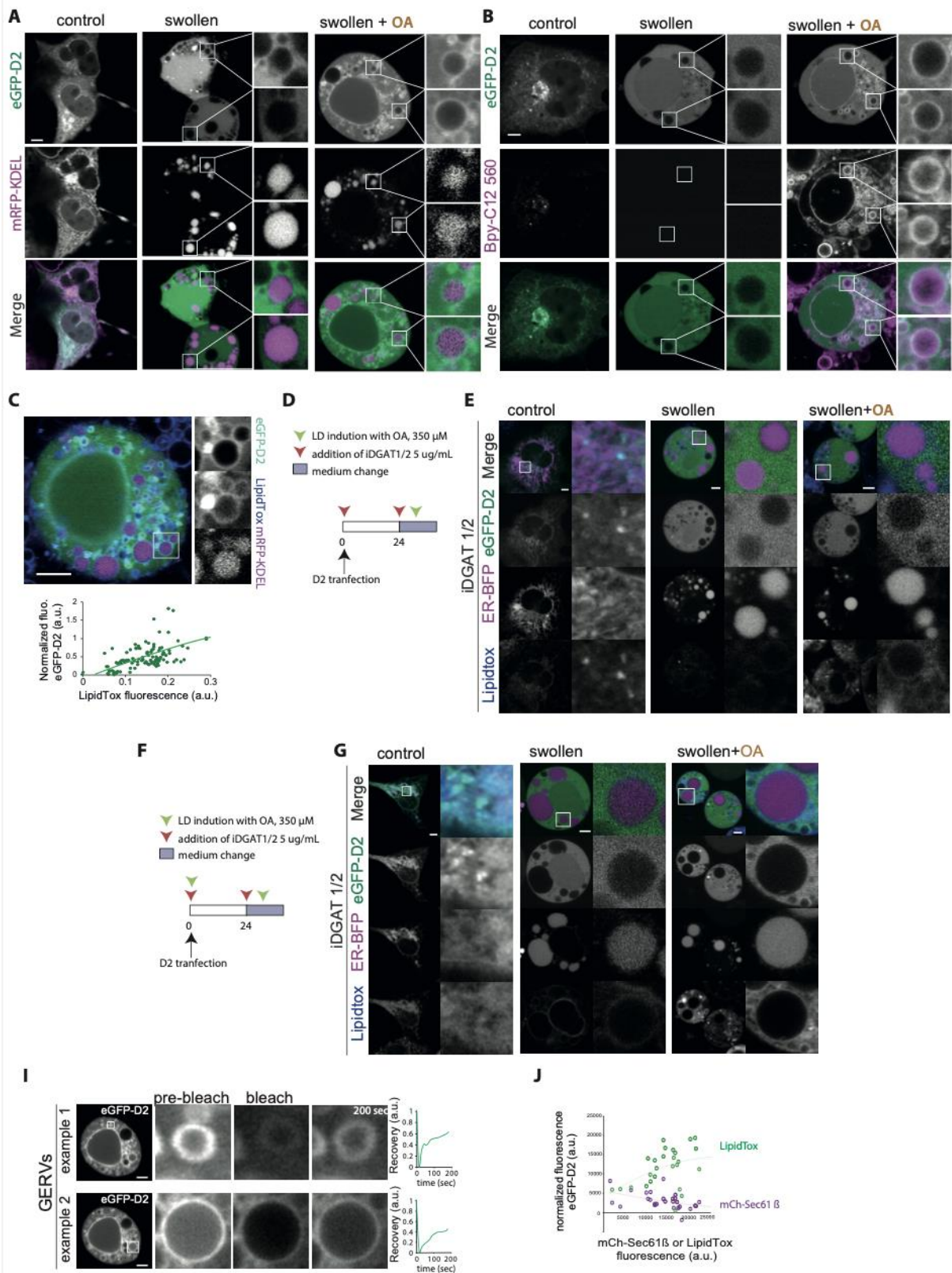


Figure S5

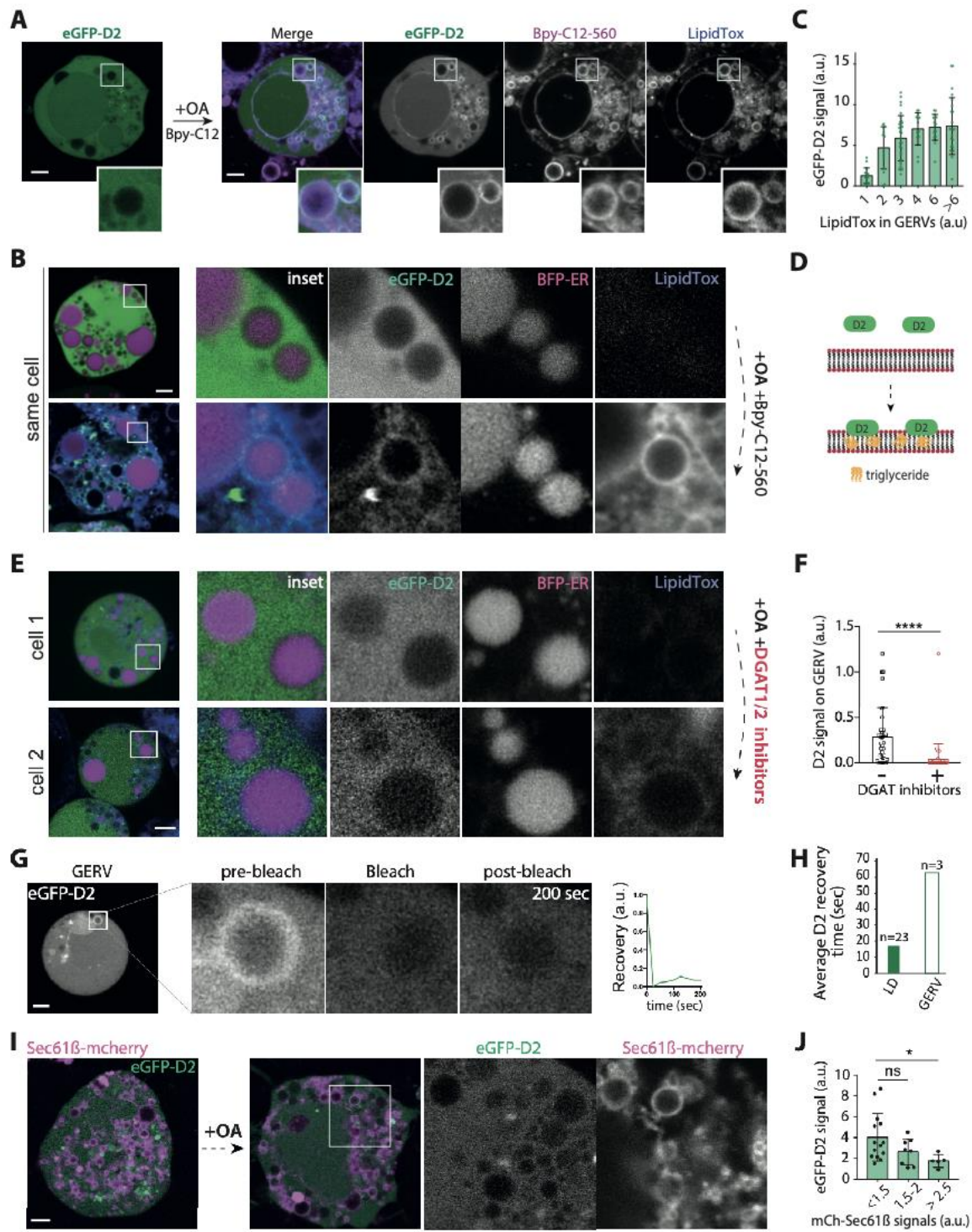


Figure 5

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