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Supplemental information

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Figure S1

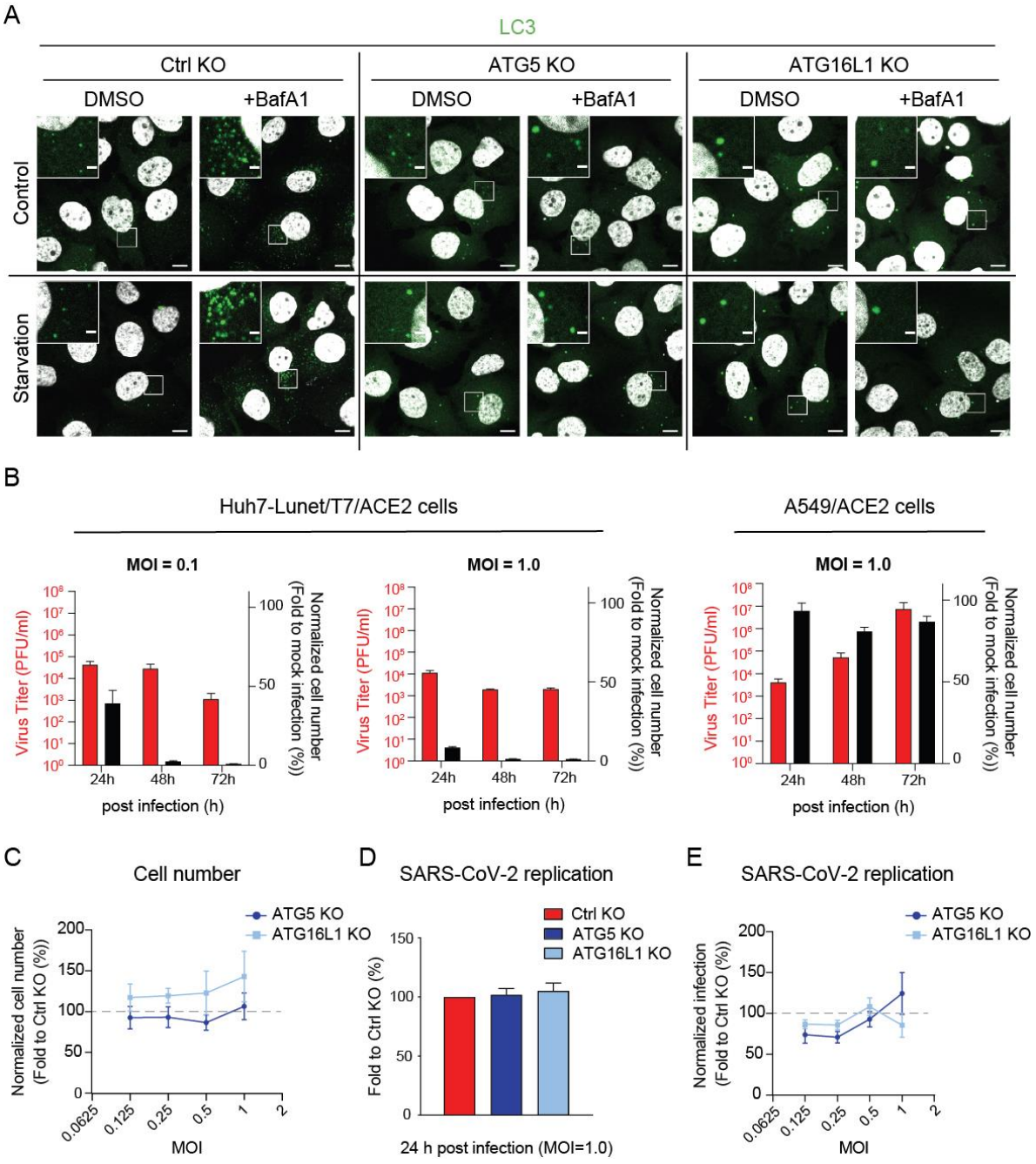

Figure S1. Functional validation of AGT5 and ATG16L1 KO and replication of SARS-CoV-2 in different cell lines including KO cell pools. Related to Figure 1. (A) Huh7-Lunet/T7 cells with ATG5 or ATG16L1 KO were subjected to starvation or cultured under regular conditions and treated or not with 100 nM Bafilomycin A1 (BafA1) for 2 h prior to fixation. Fixed cells were stained for LC3. Upper-left inserts show magnifications of white boxed areas in each image. Scale bar for overview

image, 10 µm; for magnified image, 2 µm. Quantification of a larger set of images is given in Figure 1E. (B) Replication kinetics of SARS-CoV-2 in Huh7-Lunet/T7/ACE2 and A549/ACE2 cells after infection at different MOIs. Titers of infectious virus were determined by plaque assay and cell numbers were analyzed by DAPI staining followed by CellProfiler image analysis. Data represent mean ± SEM from two independent experiments. (C-E) Huh7-Lunet/T7/ACE2 cells with given ATG KOs or control KO cells were infected with SARS-CoV-2 at indicated MOI. (C) Total cell numbers (minimum 5,000 cells per sample) were analyzed by DAPI staining followed by CellProfiler image analysis at 24 h post-infection. Values were normalized to those of infected control KO cells (set to 100% as indicated with the dotted horizontal line). Data represent mean ± SEM from two independent experiments. (D) Cells were infected as in panel (C) at MOI = 1. After 24 h, virus replication was determined by measuring the amount of SARS-CoV-2 nucleocapsid protein using immunostaining. Data represent mean ± SEM from three independent experiments. (E) Cells were infected with SARS-CoV-2 at MOIs specified on the bottom and fixed 16 h later. Virus replication was determined using nucleocapsid protein (N) staining and the percentage of N-positive cells was determined using the CellProfiler image analysis software package. Percentage infection for ATG5 or ATG16L1 KO cells was calculated by normalization of values to those obtained with control KO cells. Normalized data from 3 biologically independent experiments are plotted. Data represent mean ± SEM from two independent experiments.

Figure S2

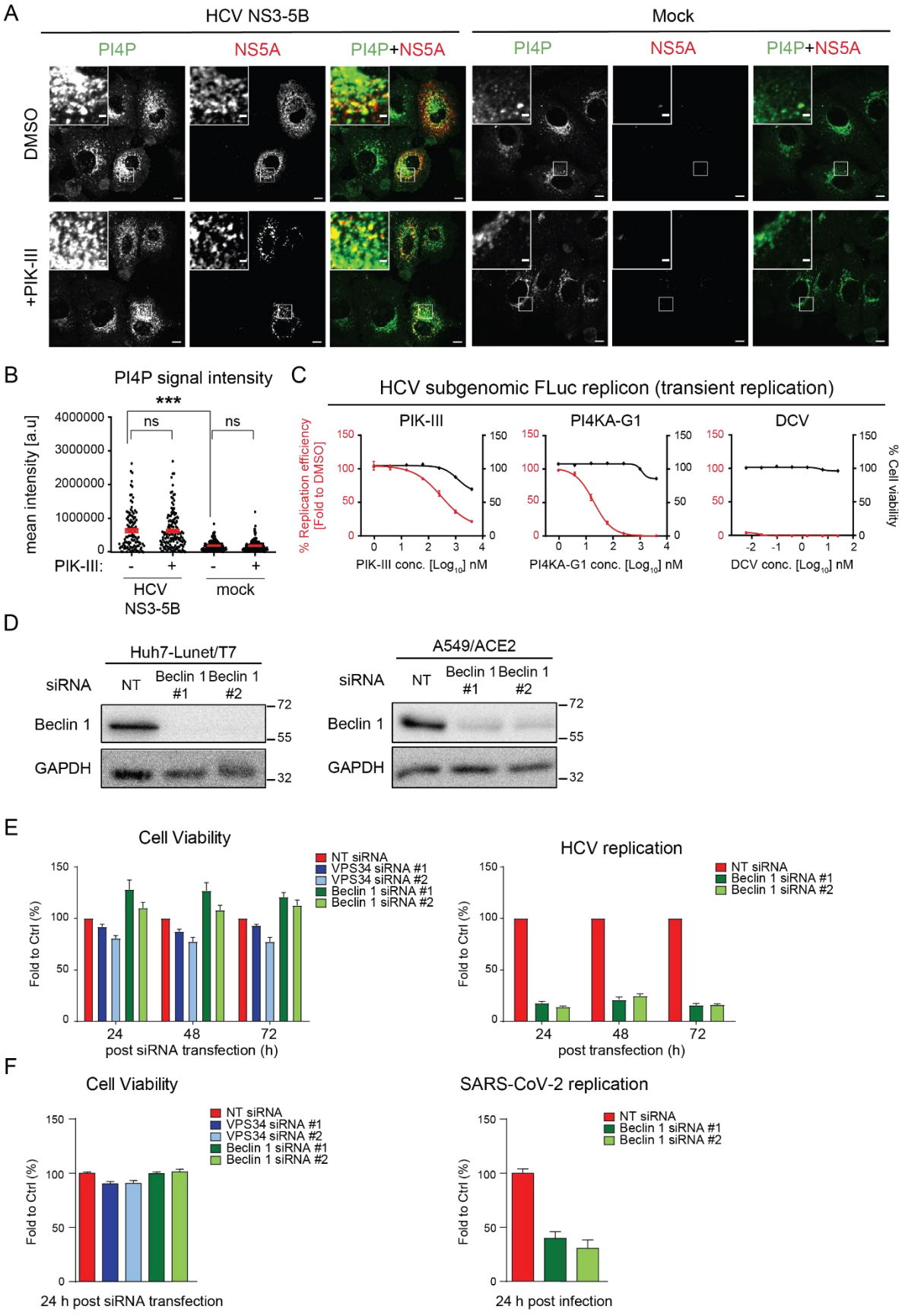
A
HCV NS3-5B
Mock
PI4P
NS5A
PI4P+NS5A
DMSO
+PIK-III
B
PI4P signal intensity
mean intensity [a.u]
PIK-III:
HCV NS3-5B
mock
C
HCV subgenomic FLuc replicon (transient replication)
PIK-III
PI4KA-G1
DCV
% Replication efficiency [Fold to DMSO]
% Cell viability
PIK-III conc. [Log10] nM
PI4KA-G1 conc. [Log10] nM
DCV conc. [Log10] nM
D
Huh7-Lunet/T7
A549/ACE2
siRNA
NT
Beclin 1 #1
Beclin 1 #2
Beclin 1
GAPDH
E
Cell Viability
HCV replication
NT siRNA
VPS34 siRNA #1
VPS34 siRNA #2
Beclin 1 siRNA #1
Beclin 1 siRNA #2
Fold to Ctrl (%)
post siRNA transfection (h)
post transfection (h)
F
Cell Viability
SARS-CoV-2 replication
24 h post siRNA transfection
24 h post infection

Figure S2. Evaluation of knockdown of the PI3K core components VPS34 and Beclin1 and impairment of HCV and SARS-CoV-2 replication upon Beclin1 depletion. Related to Figure 2.

(A-B) Pharmacological inhibition of PI3K does not affect PI4P levels. Huh7-Lunet/T7 cells were transfected with the HCV NS3-5B expression construct and on the next day treated with DMSO or 1 µM PIK-III for 2 h. Fixed cells were stained with PI4P- and NS5A-specific antibodies. (A) Upper-left inserts show magnifications of white boxed areas in each image. Scale bar for overview image, 10 µm; for magnified image, 2 µm. (B) Quantification of PI4P puncta. At least 80 cells were analyzed in each sample by using the “Analyzed Particles” function in ImageJ. Data represent mean ± SEM from two independent experiments. ***p<0.001; ns, non-significant. (C) Huh7-Lunet/T7 cells were transfected with a subgenomic HCV reporter replicon. After 4 h, cells were treated with different concentrations of drugs given on the top of each panel. Luciferase activity reflecting HCV replication efficiency and cell viability were measured at 24 h post-transfection. DCV, Daclatasvir. (D) Beclin 1 depletion by KD in Huh7-Lunet/T7 and A549/ACE2 cells was determined by western blotting. GAPDH served as loading control. (E) Left panel: Effect of VPS34 (corresponding to Figure 2C) and Beclin 1 KD on viability of Huh7-Lunet/T7 cells as determined by Celltiter Glo assay (measuring ATP content) 24, 48, and 72 h after the last siRNA transfection. Right panel: Luciferase activity reflecting HCV RNA replication was measured 24, 48, and 72 h post HCV replicon RNA transfection. (F) Left panel: Effect of VPS34 (corresponding to Figure 2G) and Beclin 1 KD on viability of A549/ACE2 cells (measuring ATP content) was determined 24 h after the last siRNA transfection. Right panel: Percentage of SARS-CoV-2 replication was determined by N-protein specific immune-staining 24 h post-infection. Data in (C), (E), and (F) represent the mean ± SEM from three independent experiments.

Figure S3

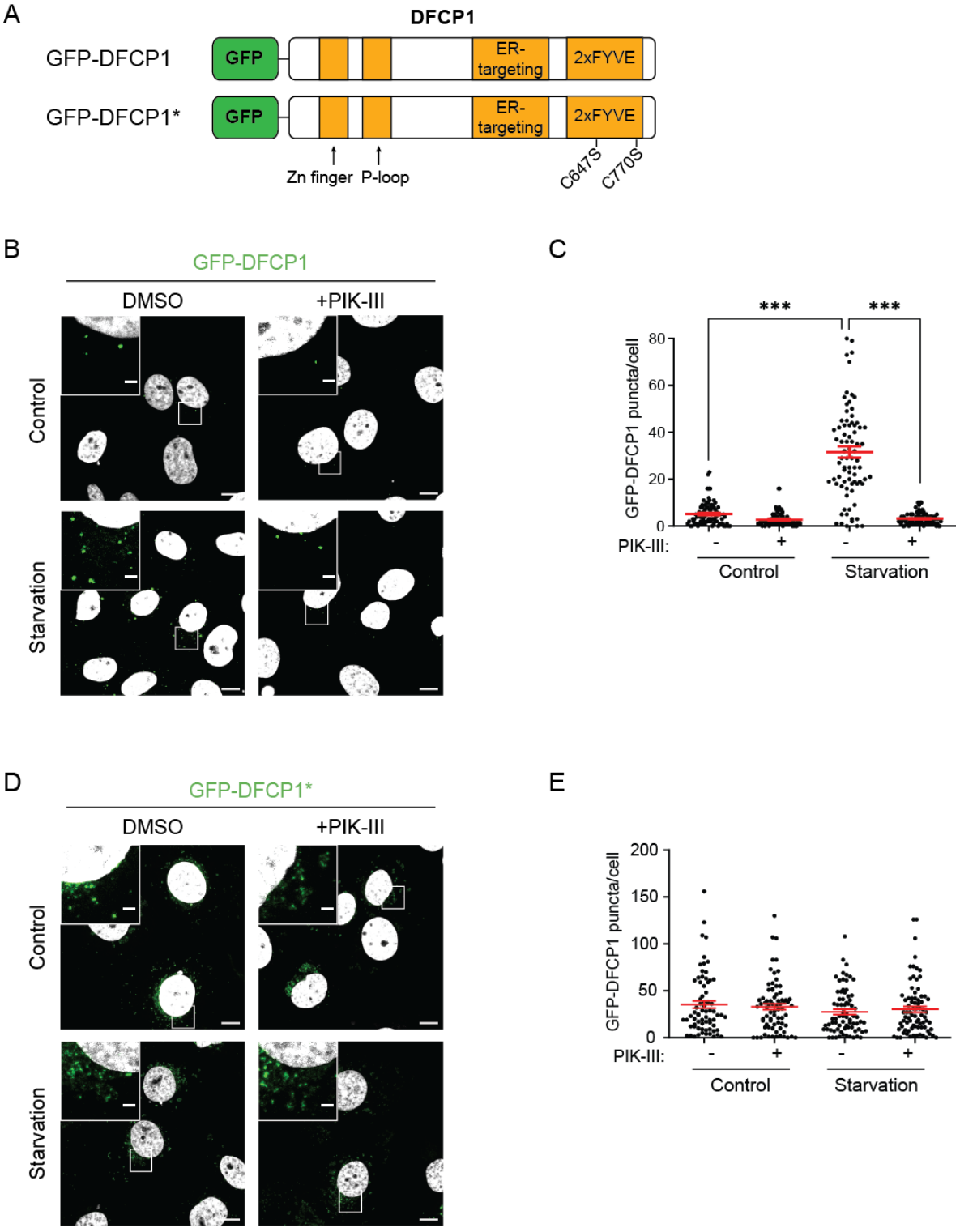
A
DFCP1
GFP-DFCP1
GFP
ER-targeting
2xFYVE
GFP-DFCP1*
GFP
ER-targeting
2xFYVE
Zn finger
P-loop
C647S
C770S
B
GFP-DFCP1
DMSO
+PIK-III
Control
Starvation
C
***
***
GFP-DFCP1 puncta/cell
80
60
40
20
0
PIK-III:
-
+
-
+
Control
Starvation
D
GFP-DFCP1*
DMSO
+PIK-III
Control
Starvation
E
GFP-DFCP1 puncta/cell
200
150
100
50
0
PIK-III:
-
+
-
+
Control
Starvation

Figure S3. Generation and validation of the intracellular PI3P sensor GFP-DFCP1. Related to Figure 3.

(A) Schematic depiction of the GFP-DFCP1 and GFP-DFCP1* (mutant) probes. The latter contains the two given amino acid substitutions disrupting PI3P binding (Axe et al., 2008). (B, C) Huh7-Lunet/T7 cell pools stably expressing GFP-DFCP1 were subjected to starvation or cultured under normal conditions (control) and treated or not with 1 µM PIK-III for 2 h. (B) Representative confocal microscopy images showing the subcellular distribution of GFP-DFCP1. Upper-left inserts show magnifications of white boxed areas. (C) Quantification of GFP-DFCP1 puncta. At least 80 cells were analyzed for each condition. (D, E) Huh7-Lunet/T7 cells stably expressing the sensor mutant GFP-DFCP1* were treated and analyzed as described for (B) and (C). Scale bar for overview image, 10 µm; for magnified image, 2 µm. All data represent mean ± SEM from three independent experiments. ***p<0.001.

93 **Figure S4**

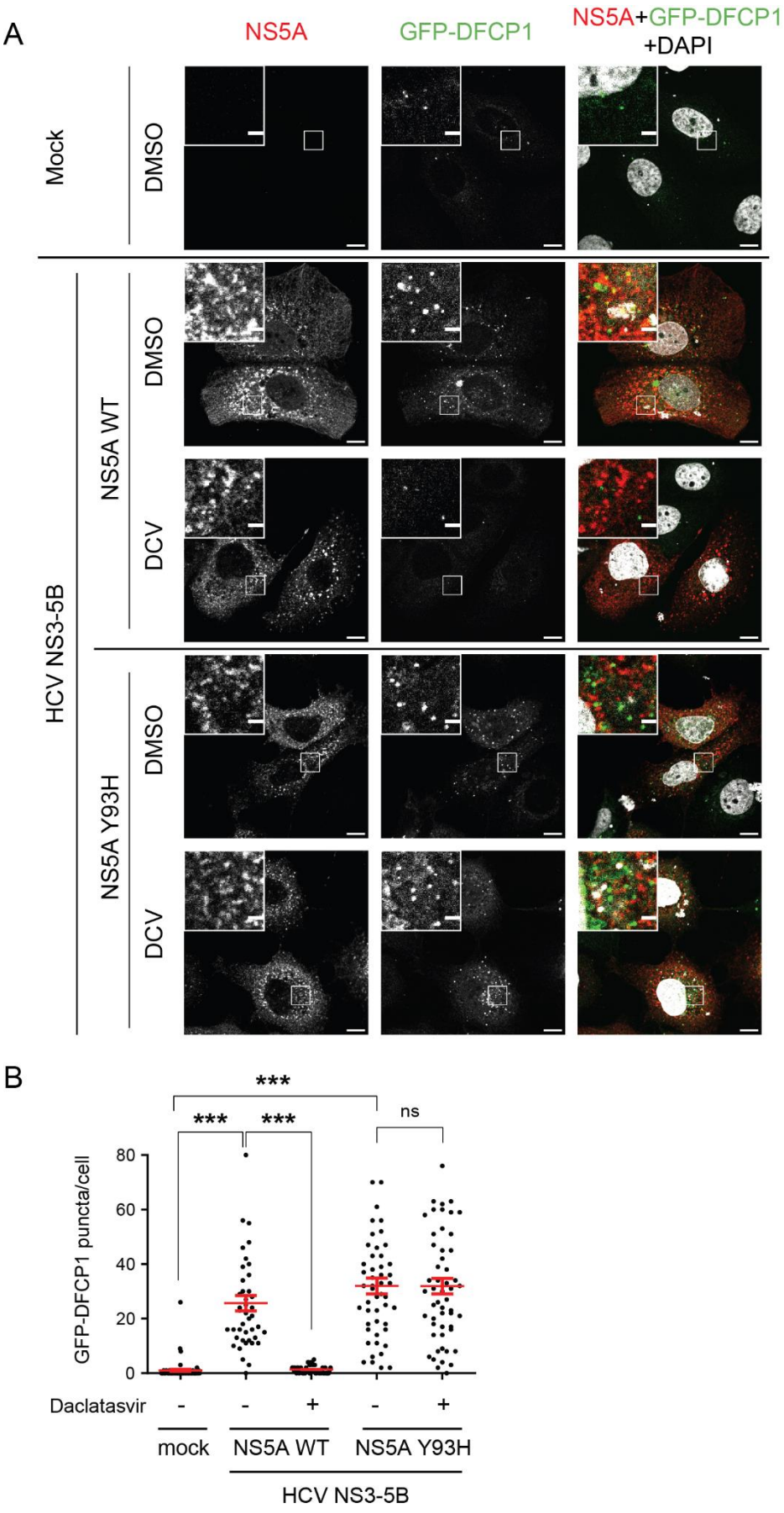
A
NS5A
GFP-DFCP1
NS5A+GFP-DFCP1
+DAPI
Mock
DMSO
HCV NS3-5B
NS5A WT
DMSO
DCV
NS5A Y93H
DMSO
DCV
B
***
***
***
ns
GFP-DFCP1 puncta/cell
80
60
40
20
0
Daclatasvir
-
-
+
-
+
mock
NS5A WT
NS5A Y93H
HCV NS3-5B

Figure S4. NS5A is required for HCV NS3-5B induced increase of PI3P. Related to Figure 3.

Huh7-Lunet/T7 cells stably expressing GFP-DFCP1 were transfected with the HCV NS3-5B expression plasmid encoding either NS5A wildtype (WT) or NS5A Y93H, a mutation conferring high-level resistance to the NS5A inhibitor Daclatasvir (DCV). After 5 h, cells were treated with 1 nM DCV and cells were fixed 24 h after transfection. (A) Representative confocal microscopy images showing the subcellular distribution of GFP-DFCP1 and NS5A. Upper-left inserts show magnifications of white boxed areas. (B) Quantification of GFP-DFCP1 puncta. At least 50 cells were analyzed for each condition. Scale bar for overview image, 10 µm; for magnified image, 2 µm. Data represent mean ± SEM from two independent experiments. ***p<0.001; ns, no significant.

Figure S5

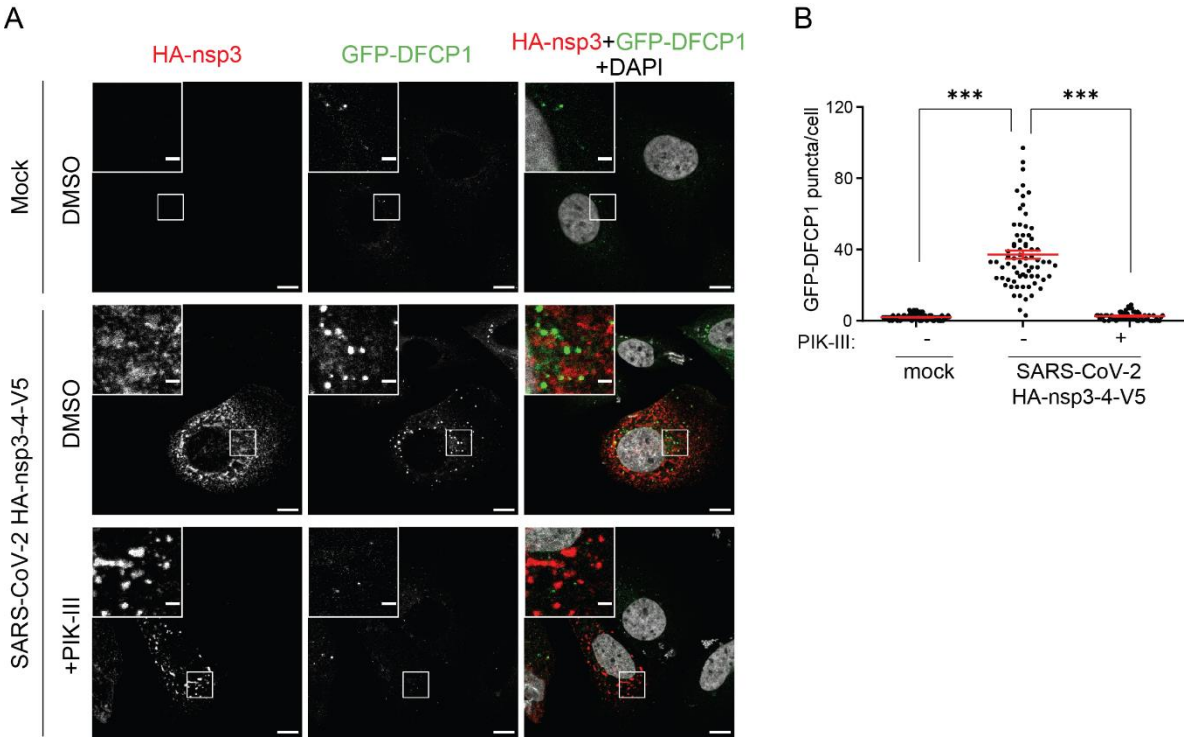

Figure S5. SARS-CoV-2 nsp3-4 expression increases intracellular PI3P levels.

Related to Figure 3.

Huh7-Lunet/T7 cells stably expressing GFP-DFCP1 were transfected with the SARS-CoV-2 HA-nsp3-4-V5 expression construct to induce the formation of DMVs. After 24 h, cells were treated with 1 µM PIK-III for 2 h prior to fixation. (A) Representative confocal microscopy images showing the subcellular distribution of GFP-DFCP1 and SARS-CoV-2 HA-nsp3. Upper-left inserts show magnifications of white boxed areas. (B) Quantification of GFP-DFCP1 puncta. At least 60 cells were analyzed for each condition. Scale bars for overview images, 10 µm; for magnified images, 2 µm. Data represent mean ± SEM from two independent experiments. ***p<0.001.

Figure S6

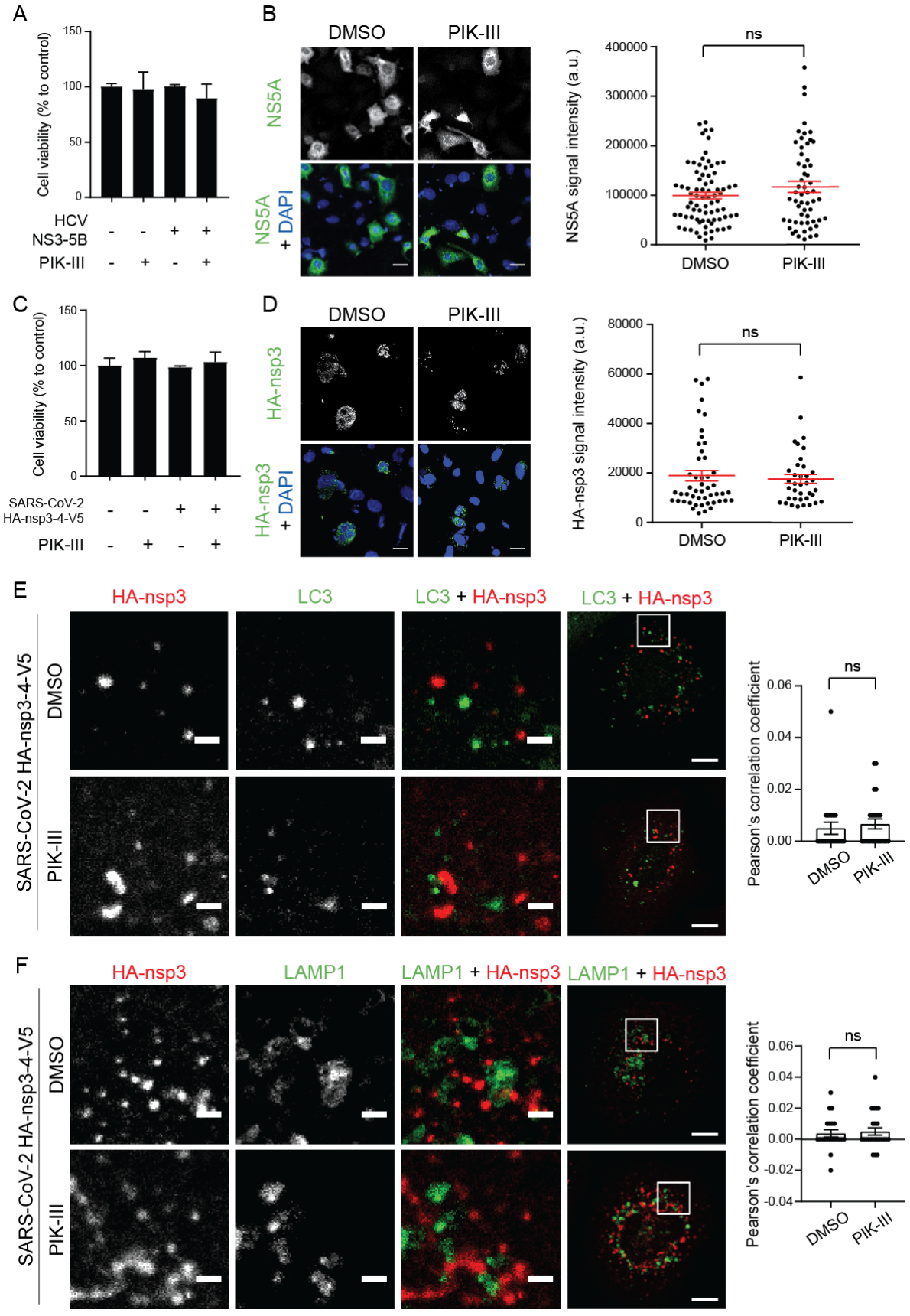
A
Cell viability (% to control)
150
100
50
0
HCV
NS3-5B
PIK-III
B
DMSO
PIK-III
NS5A
NS5A + DAPI
NS5A signal intensity (a.u.)
400000
300000
200000
100000
0
ns
DMSO
PIK-III
C
Cell viability (% to control)
SARS-CoV-2
HA-nsp3-4-V5
PIK-III
D
HA-nsp3
HA-nsp3 + DAPI
HA-nsp3 signal intensity (a.u.)
80000
60000
40000
20000
E
HA-nsp3
LC3
LC3 + HA-nsp3
SARS-CoV-2 HA-nsp3-4-V5
DMSO
PIK-III
Pearson's correlation coefficient
0.06
0.04
0.02
0.00
F
LAMP1
LAMP1 + HA-nsp3
-0.02
-0.04

Figure S6. No evidence for enhanced degradation of HCV NS3-5B and SARS-CoV-2 nsp3-4 upon pharmacological inhibition of PI3K. Related to Figure 4.

(A-B) Huh7-Lunet/T7 cells were transfected with the HCV NS3-5B expression plasmid. After 5 h, 1 µM PIK-III was added to the cells that were lysed or fixed 24 h after transfection to measure cell viability or NS5A, respectively. (A) Cell viability was determined using WST-1 assay. Data represent mean ± SEM from two independent experiments. (B) Fixed cells were stained for NS5A and nuclear DNA was stained with DAPI. NS5A signal intensity from 5 randomly selected areas was quantified by using ImageJ. Scale bar, 30 µm. (C-F) Huh7-Lunet/T7 cells were transfected with the SARS-CoV-2 HA-nsp3-4-V5 expression plasmid and 24 h later, 1 µM PIK-III was added to the cells. After 48 h, cells were lysed or fixed for further analysis. (C) Cell viability was determined by using WST-1 assay. (D) Fixed cells were stained for HA-nsp3 by using a HA-specific antibody and signal intensity from 5 randomly selected areas was quantified using ImageJ. Scale bar, 30 µm. Data in (A-D) represent mean ± SEM. In (B) and (D), ns, not significant, according to two tailed, unpaired Student's t-test. (E, F) Fixed cells were stained for (E) HA-nsp3 and LC3, or (F) HA-nsp3 and LAMP1. The boxed area in the overview panel in the right indicates the magnified region that is displayed in the other panels. Scale bars in the overview and enlarged section represent 10 µm and 2 µm, respectively. The degrees of colocalization between HA-nsp3 and LC3, or LAMP1 and LC3, were quantified by determining Pearson's correlation coefficients. Analyses are based on at least 20 cells per condition. Data in (E) and (F) represent mean ± SEM from two independent experiments. ns, not significant, according to two tailed, unpaired Student's t-test.

Table S1. Oligonucleotides. Related to STAR METHODS.

Oligonucleotide name: Sequence	Source	Identifier
siRNA: NT Control	Life Technologies	4390846
siRNA: VPS34 #1: GCUUAGACCUGUCGGAUGATT	Life Technologies	s10519
siRNA: VPS34 #2: GCAUGGAGAUGAUUUACGUTT	Life Technologies	s10518
siRNA: Beclin 1 #1: CAGUUACAGAUGGAGCUUAATT	Life Technologies	s16537
siRNA: Beclin 1 #2: CAGAUACUCUUUUAGACCATT	Life Technologies	s16539
siRNA: DFCP1 #1: GGAUGGGUCUCGCAAAAUATT	Life Technologies	s28712
siRNA: DFCP1 #2: GGAUGUAAGAAAAGCAUGATT	Life Technologies	s28713
PCR primer: GFP-DFCP1_BamHI_F: AAAAGGATCCGCCACCATGGTGAGCAAGGGCGAG	Merck, Darmstadt, Germany	N/A
PCR primer: GFP-DFCP1_NotI_R: AAAAGCGGCCGCTTAAAGGTCACCGGGCTTTTTTATTG	Merck, Darmstadt, Germany	N/A
PCR primer: DFCP1_FYVE*1_F: AGTCGGTGTCCGAGCTTAGCCTTGGACCCACCAAGG	Merck, Darmstadt, Germany	N/A
PCR primer: DFCP1_FYVE*1_R: CCTGGCTTCGTAGCTGTTGTCACAGAC	Merck, Darmstadt, Germany	N/A
PCR primer: DFCP1_FYVE*2_F: TGACAACAGCTACGAAGCCAGGAACG	Merck, Darmstadt, Germany	N/A
PCR primer: DFCP1_FYVE*2_R: TGCGGCCGCTTAAAGGTCACCGGGCTTTTTATTGCTGTTG	Merck, Darmstadt, Germany	N/A
PCR primer: piRO_SARS2_F: CACCTGATAATCTAGATAAGCACCAATCTTAGTGTTG	Merck, Darmstadt, Germany	N/A
PCR primer: piRO_SARS2_R: TGGCACGCGTGAATTCGGGCCCGGGATTTTCCT	Merck, Darmstadt, Germany	N/A