Figure S1. Related to Figure 1

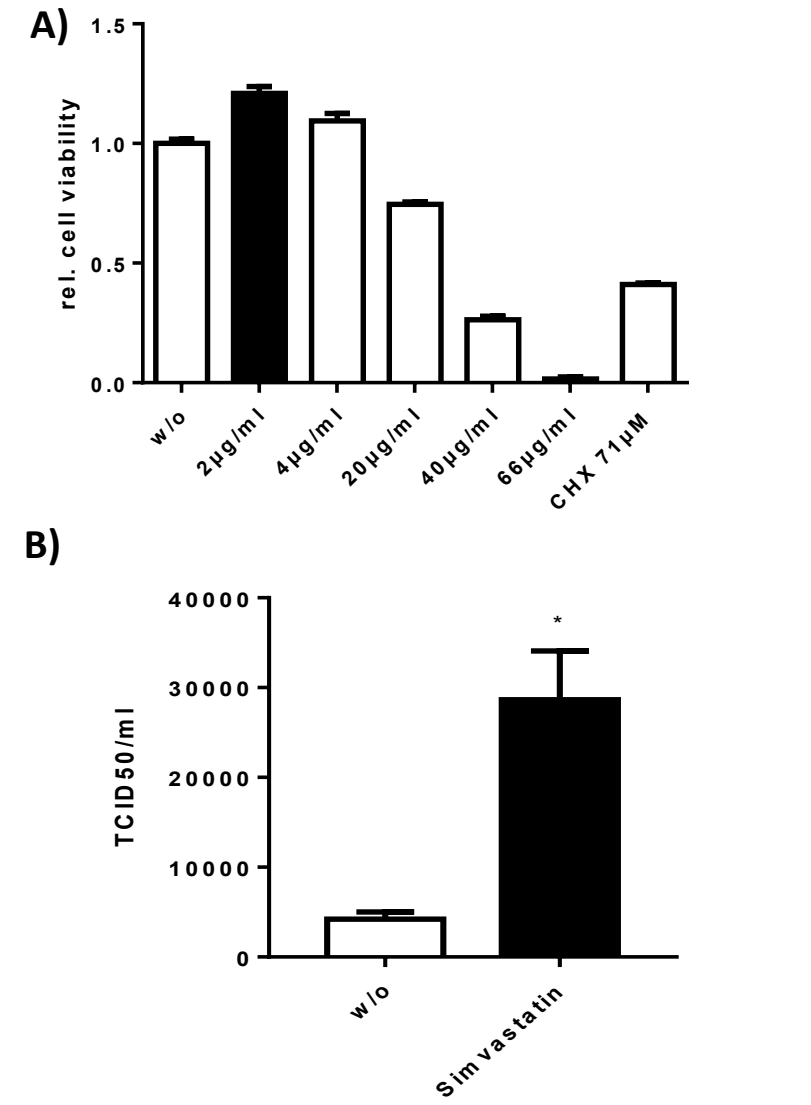


Fig. S1: Cytotoxicity,of U18666A, simvastatin increases release and core purification of supernatant

a) Huh7.5 cells were treated in a 96 well plate with the indicated concentrations of U18666A for 16 h. The cell viability was assessed by PrestoBlue assay. U18666A is toxic at high concentrations but 2 µg/ml is well tolerated and highlighted in black. Cycloheximide treatment served as positive control.

b) TCID50 of the supernatant of Huh7.5 J6 cells untreated or treated with 10 µM Simvastatin.

c) HCV Core Western Blot of heparin purified cell culture supernatant of untreated and U18666A-treated Huh7.5 J6. Cell lysates of Huh7.5 cells electroporated with J6 or GND served as positive and negative controls.

Figure S2. related to Figure 4

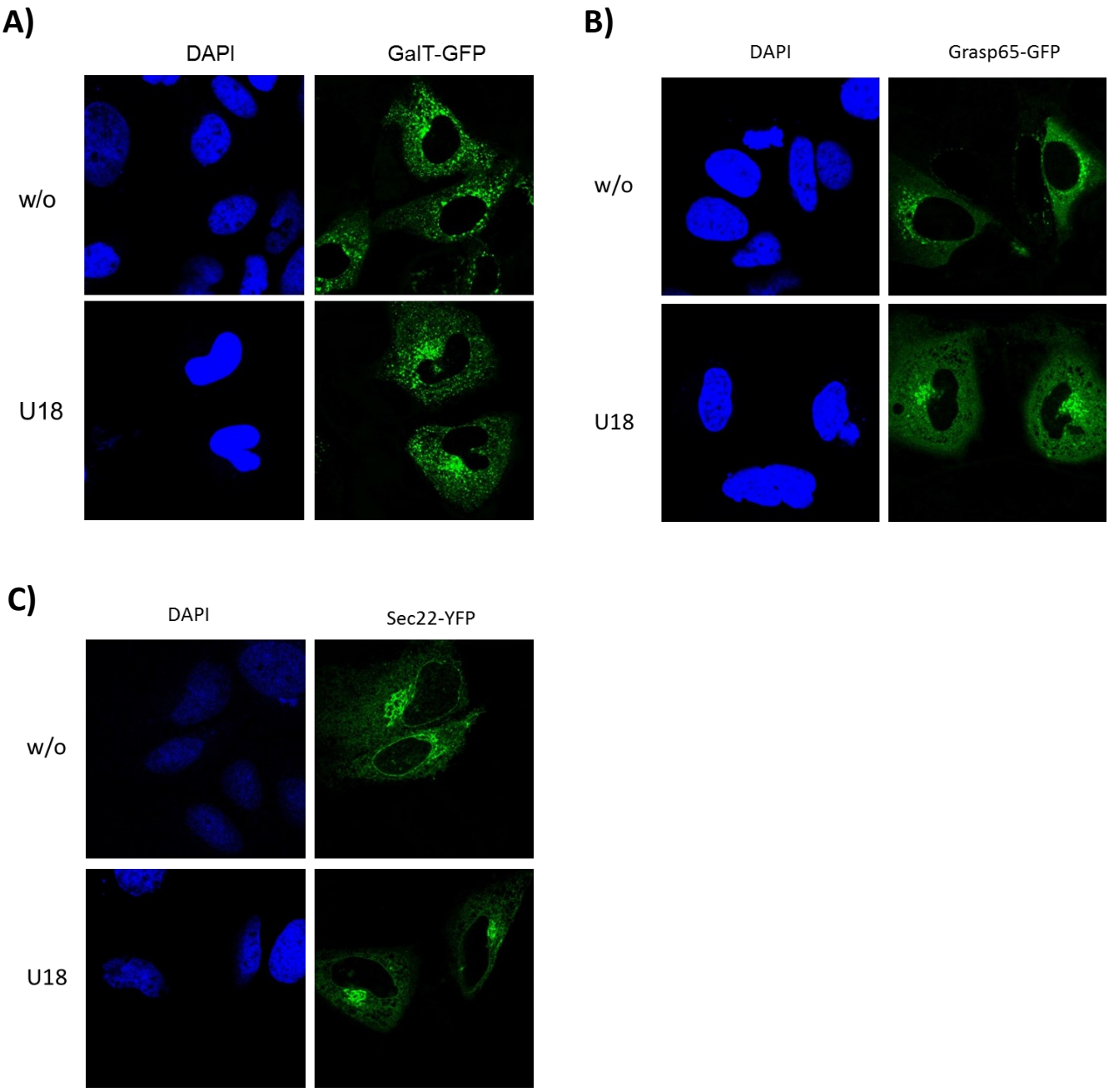


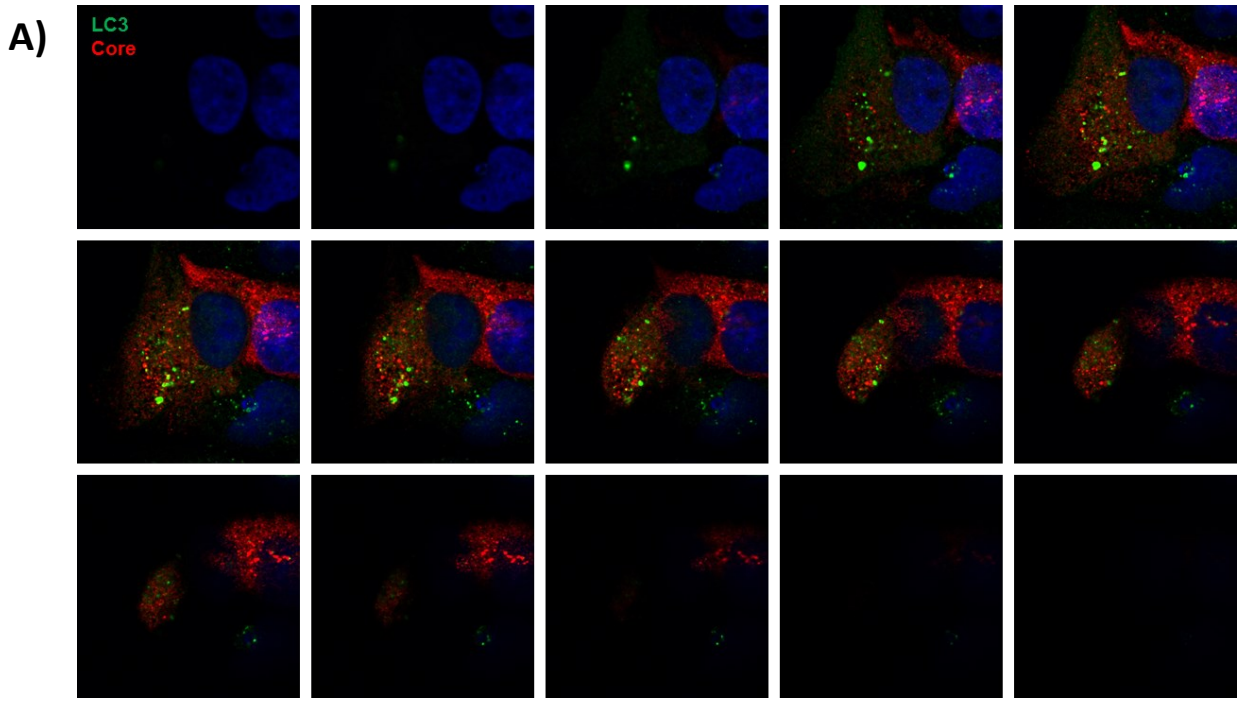
Fig. S2: U18666A does not influence the canonical secretory pathway

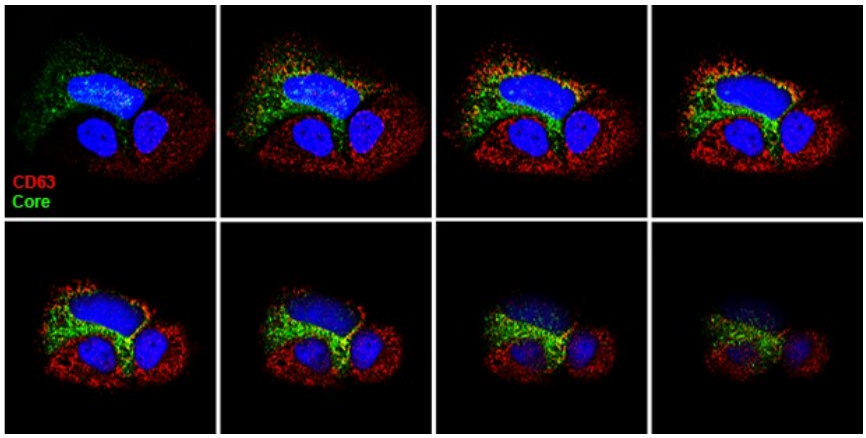
a) Huh7.5 cells were transfected with a plasmid coding for a GalT-GFP fusionprotein and treated with 2 µg/ml U18666A. The distribution of the marker protein was analyzed by CLSM and compared to untreated cells.

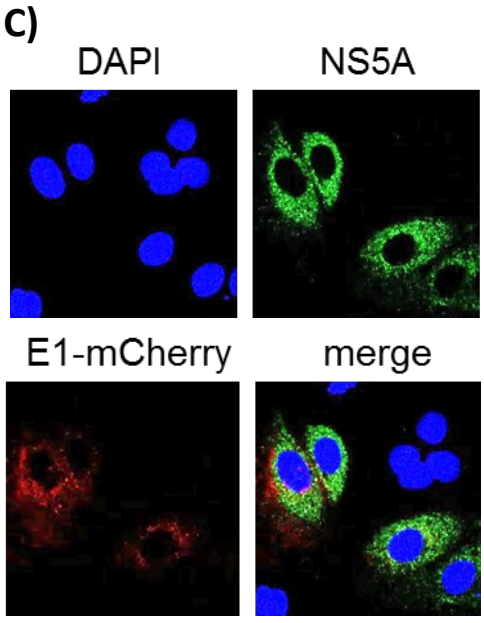
b) Huh7.5 cells were transfected with a plasmid coding for a Gasp65-GFP fusionprotein and treated with 2 µg/ml U18666A. The distribution of the marker protein was analyzed by CLSM and compared to untreated cells.

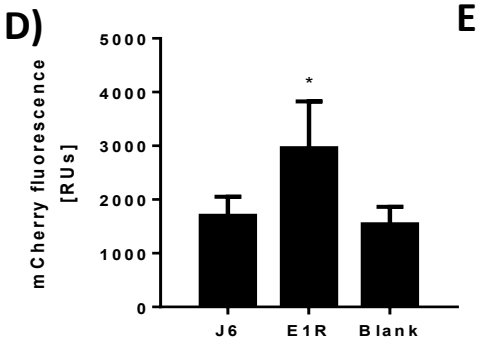
c) Huh7.5 cells were transfected with a plasmid coding for a Sec22-YFP fusionprotein and treated with 2 µg/ml U18666A. The distribution of the marker protein was analyzed by CLSM and compared to untreated cells.

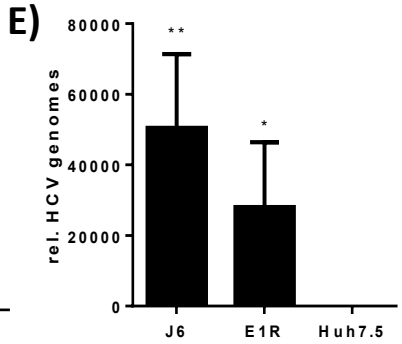
Figure S3. related to Figure 5

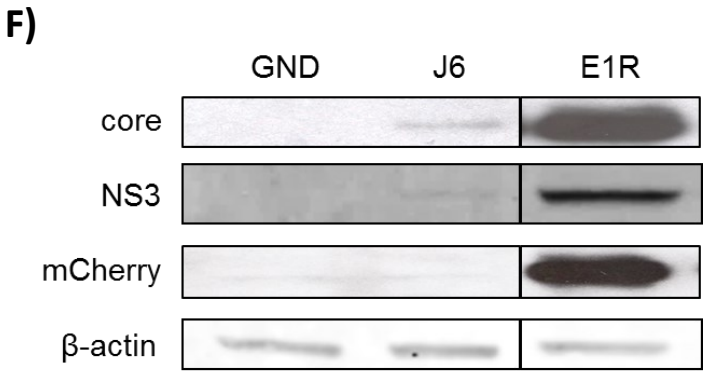












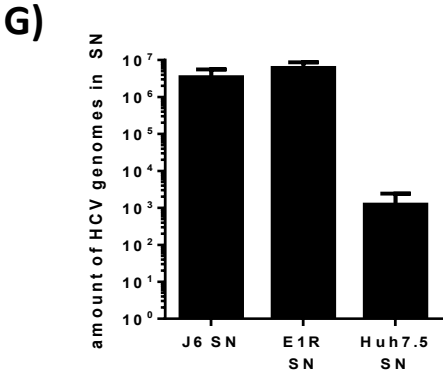


Fig S3: Characterization of Jc1-E1-mCherry and z-stacks

a) z-stack reconstruction of U18666A-treated Huh7.5 J6 cells stained against HCV core (red) and LC3 (green). The nuclei are visualized in blue.

b) z-stack reconstruction of U18666A-treated Huh7.5 J6 cells stained against HCV core (green) and CD63 (red). The nuclei are visualized in blue.

c)- g) Huh7.5 cells were electroporated with Jc1-E1-mCherry RNA (E1R) and harvested after 72h.

c) CLSM analysis shows red fluorescence in NS5A-positive cells. NS5A was stained with a specific antiserum and is visualized in green. The nuclei were stained with DAPI in blue.

d) mCherry fluorescence of cells seeded in a 96 well plate and covered with PBS. Fluorescence was measured with a microplate fluorescence reader.

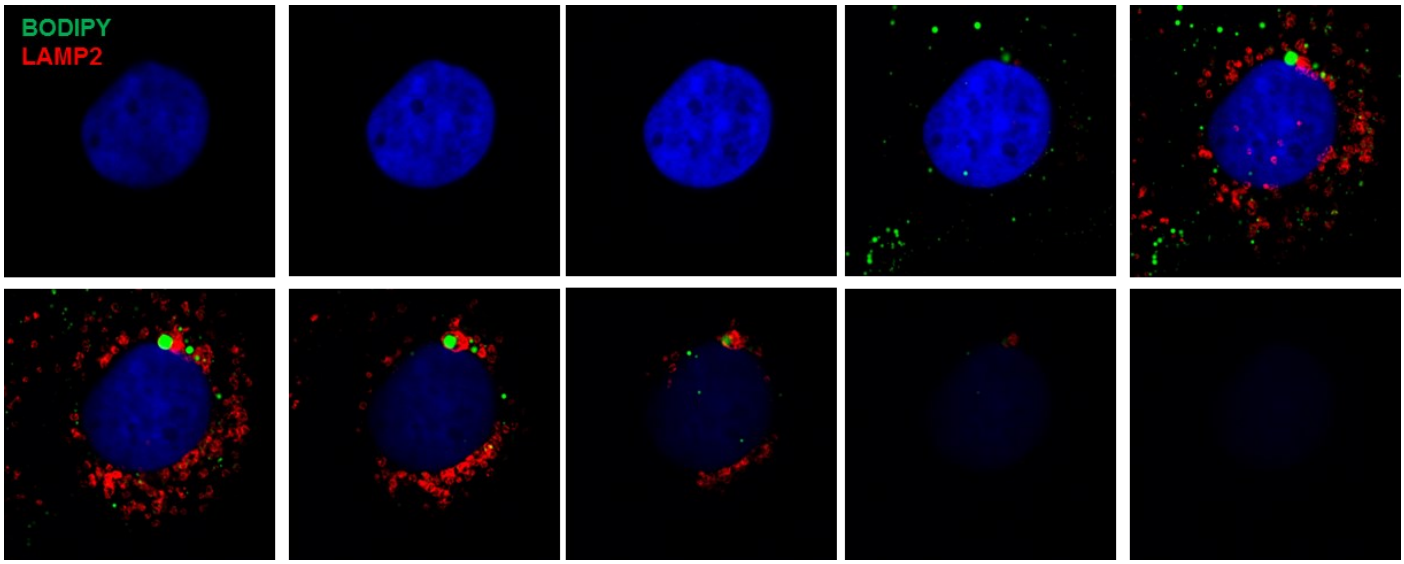
e) Jc1-E1-mCherry is able to replicate in Huh7.5 cells. The viral replication was assessed by rtPCR of the intracellular RNA.

f) rtPCR of the supernatants shows that viral genomes are released in a comparable amount to J6 replicating cells.

g) Western blot analysis confirmed the presence of HCV proteins and the mCherry-fusion protein E1 in cells electroporated with the Jc1-E1-mCherry construct.

Figure S4. related to Figure 6

A)



B)

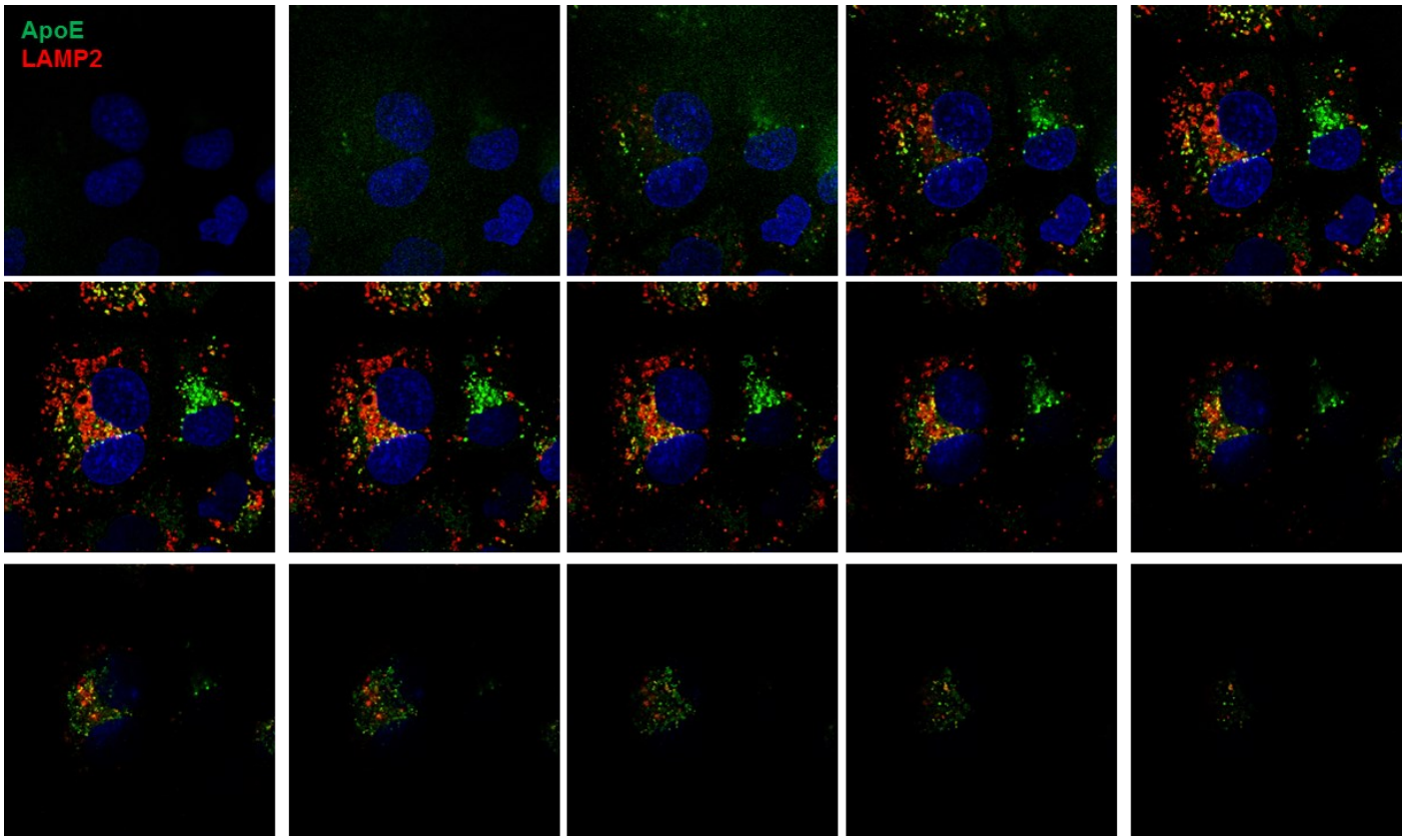


Fig S4: Z-stacks of LAMP2/BODIPY and LAMP2/ApoE

a) z-stack reconstruction of U18666A-treated Huh7.5 J6 cells stained for LAMP2 (red). The nuclei are visualized in blue. Lipids are stained with BIDIPY in green.

b) z-stack reconstruction of U18666A-treated Huh7.5 J6 cells stained against LAMP2 (red) and ApoE (green). The nuclei are visualized in blue.