Movie S1: Live tracking of Topfluor-Cholesterol (TFC, green) and NS5A-mCherry (red) in Huh7/LunetCD81H cells transfected with the subgenomic replicon containing mCherry tagged NS5A and pulsed with 1 μM Topfluor-Cholesterol early in replication. Huh7/LunetCD81H cells were transfected with the subgenomic replicon (NS3-3′, JFH1, gt2a) encoding a mCherry tagged NS5A. 30 h later cells were pulsed for 10 min with 1 μM TFC, followed by extensive washing with fresh imaging media and imaging for 16 h, with an image taken every 15 min. In addition the volume of the cell was imaged by taking three sequential z-stacks. The TFC signal and NS5A-mCherry signal after background subtraction is shown. Signals were enhanced by auto-contrast.

Movie S2: Live tracking of Topfluor-Cholesterol (TFC, green) and NS5A-mCherry (red) in Huh7/LunetCD81H cells transfected with the subgenomic replicon containing mCherry tagged NS5A and pulsed with 1 μM Topfluor-Cholesterol late in replication. Huh7/LunetCD81H cells were transfected with the subgenomic replicon (NS3-3′, JFH1, gt2a) encoding a mCherry tagged NS5A. 53 h later cells were pulsed for 10 min with 1 μM TFC, followed by extensive washing with fresh imaging media and imaging for 16 h, with an image taken every 15 min. In addition the volume of the cell was imaged by taking three sequential z-stacks. The TFC signal and NS5A-mCherry signal after background subtraction is shown. Signals were enhanced by auto-contrast.