# Stearoyl-CoA desaturase inhibition blocks formation of hepatitis C virus-induced specialized membranes

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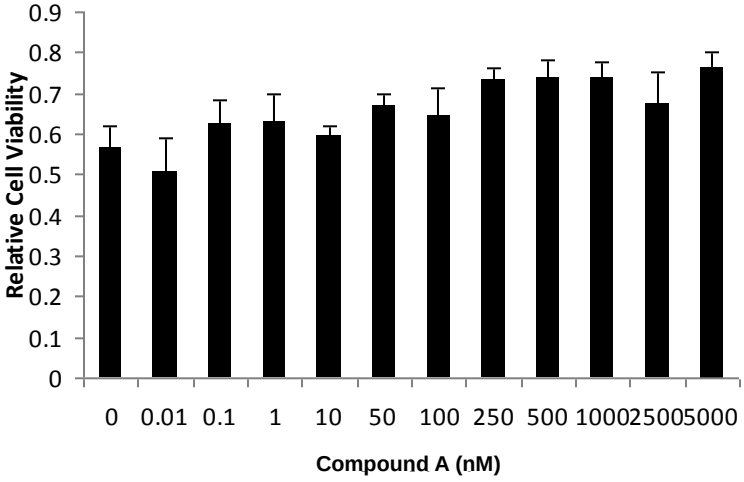
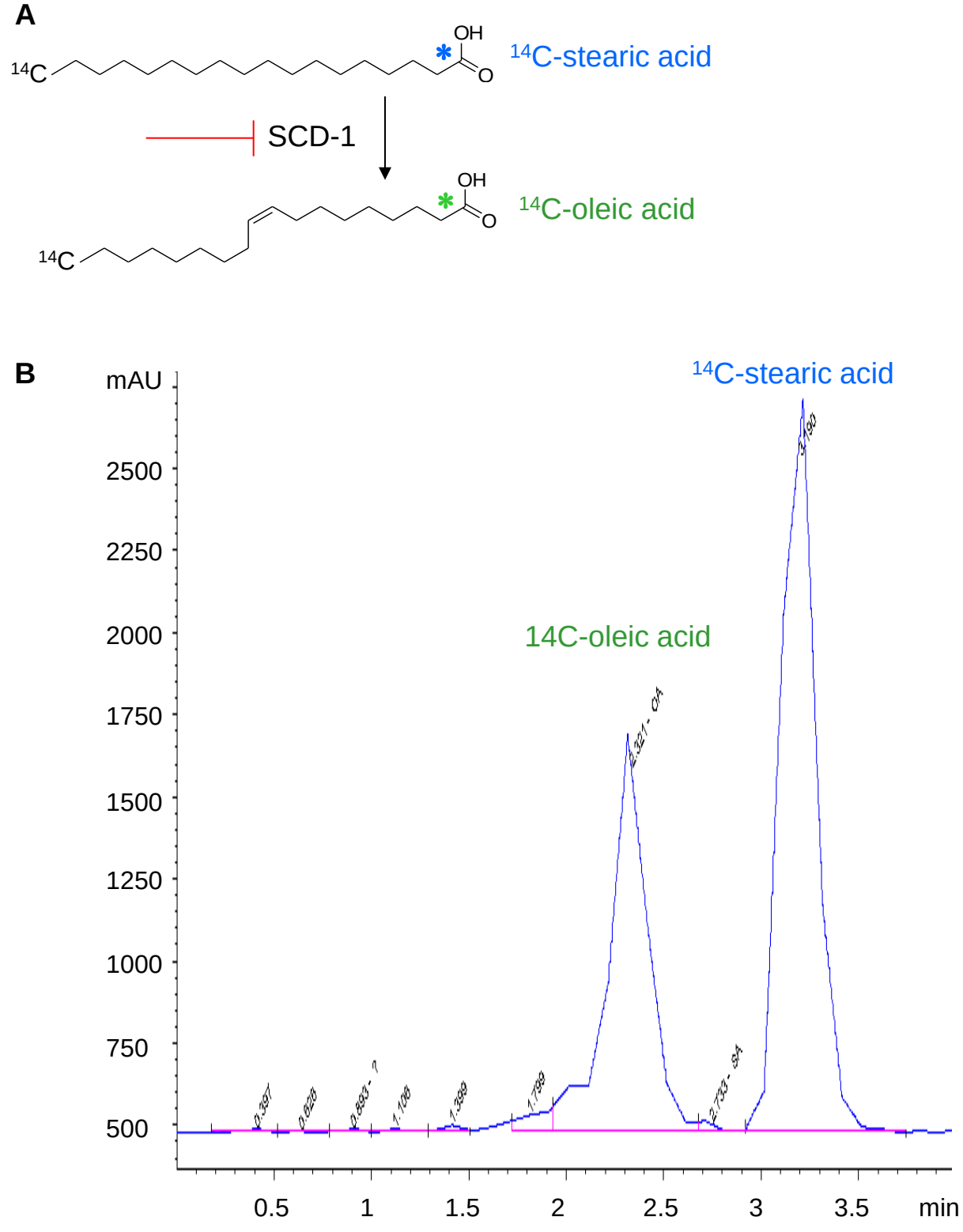
# Supplementary Figure S1



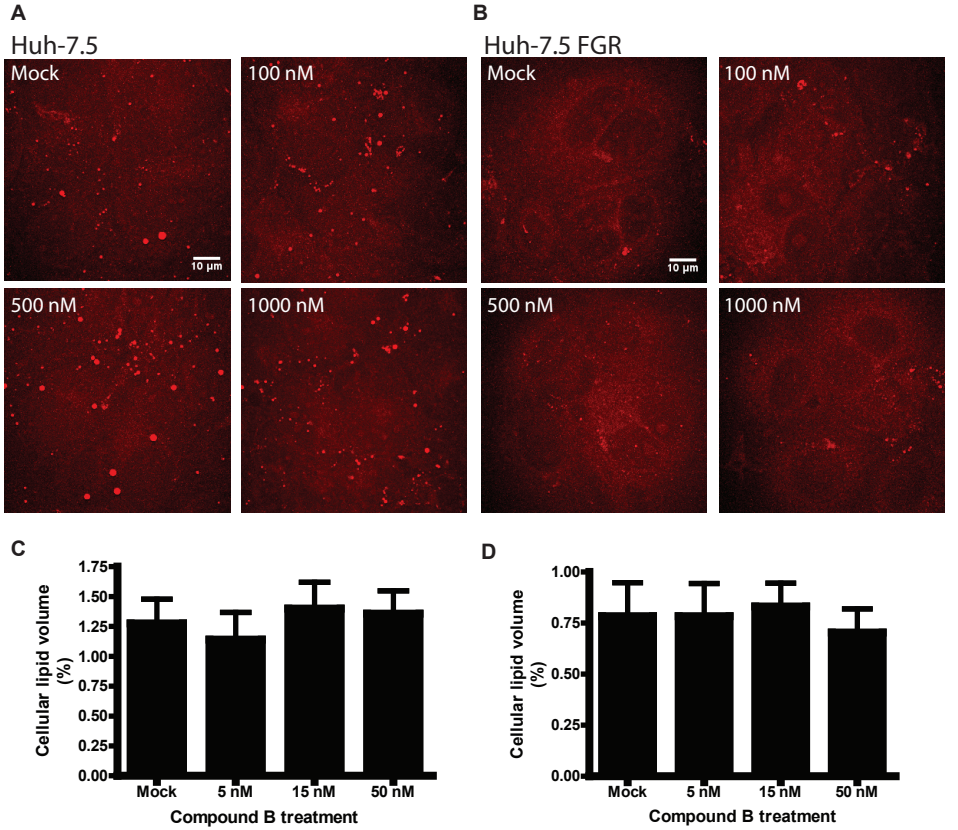

**Supplementary Figure S1: Cytotoxicity assay for inhibitor A.** The toxicity of inhibitor **A** was evaluated via an MTT assay following a 96 hour treatment in Huh7-SGR cells. Results confirm no significant cytotoxicity was observed at the concentrations tested.

# Supplementary Figure S2



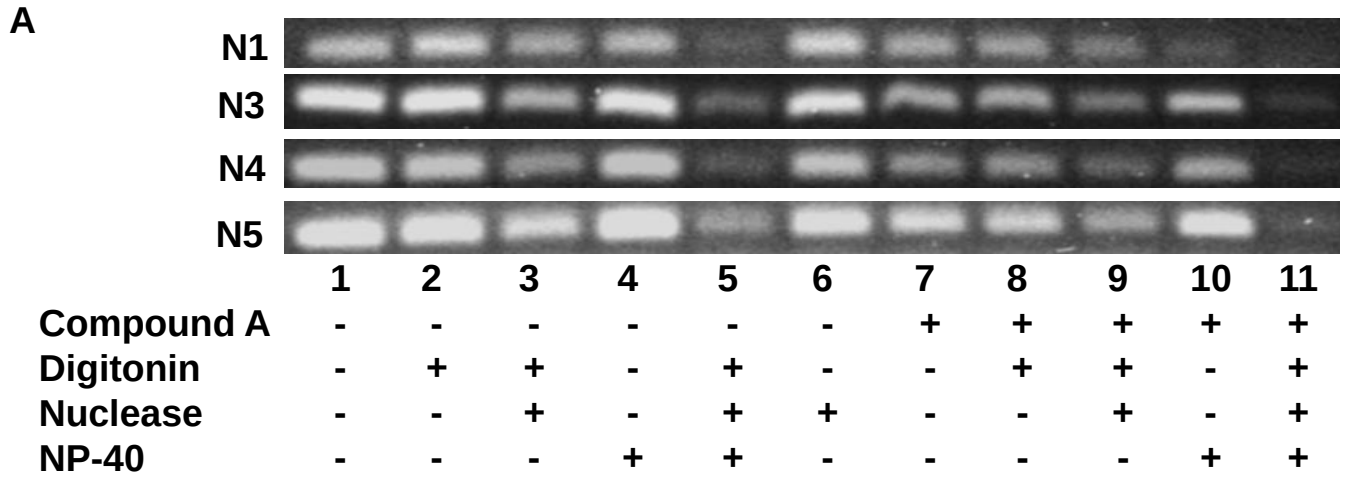

**Supplementary Figure S2: Measuring SCD-1 cell-based enzymatic activity by $^{14}C$ labeling of stearic acid**. (**a**) SCD-1 enzymatic assays were performed by treatment of cells with $^{14}C$ stearic acid. An organic extraction was performed to isolate lipids, and the conversion rate to $^{14}C$ oleic acid was subsequently measured via reverse phase HPLC. Detection was performed using a scintillation analyzer. (**b**) An illustration of a typical chromatogram is shown.

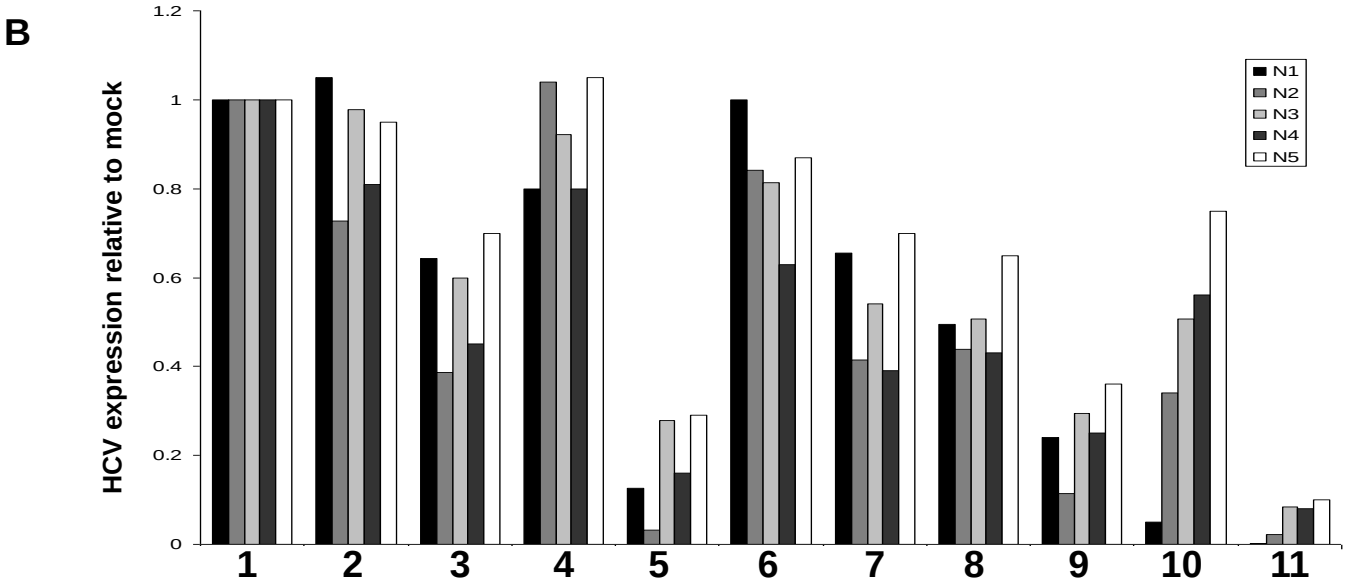
Supplementary Figure S3

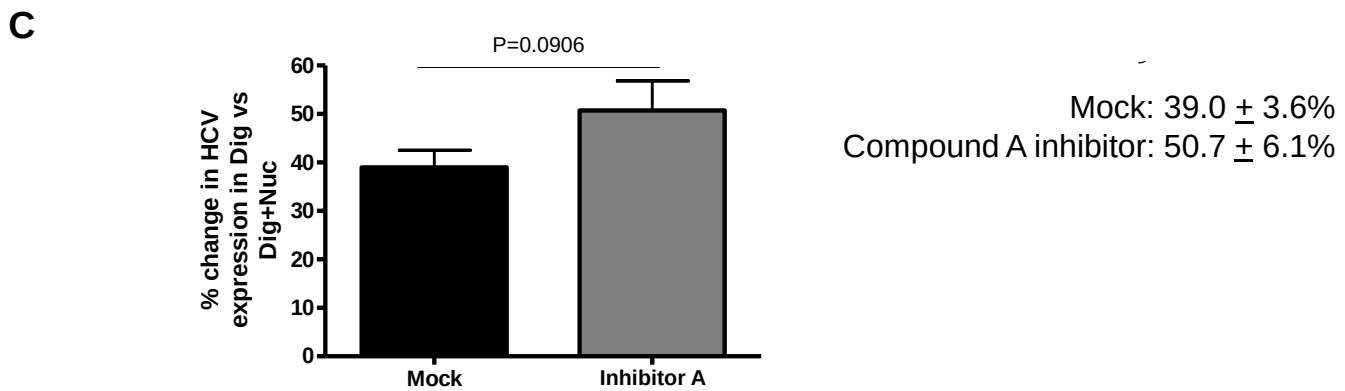



**Supplementary Figure S3: NS3 protease inhibitor has no effect on LD phenotype.** (**a**) Huh7.5 and (**b**) Huh7.5-FGR cells were treated with various concentrations of inhibitor **B**: 50nM, 15nM, and 5nM and non-treated (mock) of the inhibitor **B**. 96 hours post-treatment, cells were fixed and imaged by CARS microscopy. Representative images are shown for each concentrations (n=2). Scale bar = 10µm. The LD density was measured by voxel analysis under multiple field of views for (**c**) Huh7.5 cells (n>10) and (**d**) Huh7.5-FGR cells (n>10). The bar graphs illustrate the average cellular lipid volume measured by voxel analysis with error bars representing the standard error of the mean.

Supplementary Figure S4

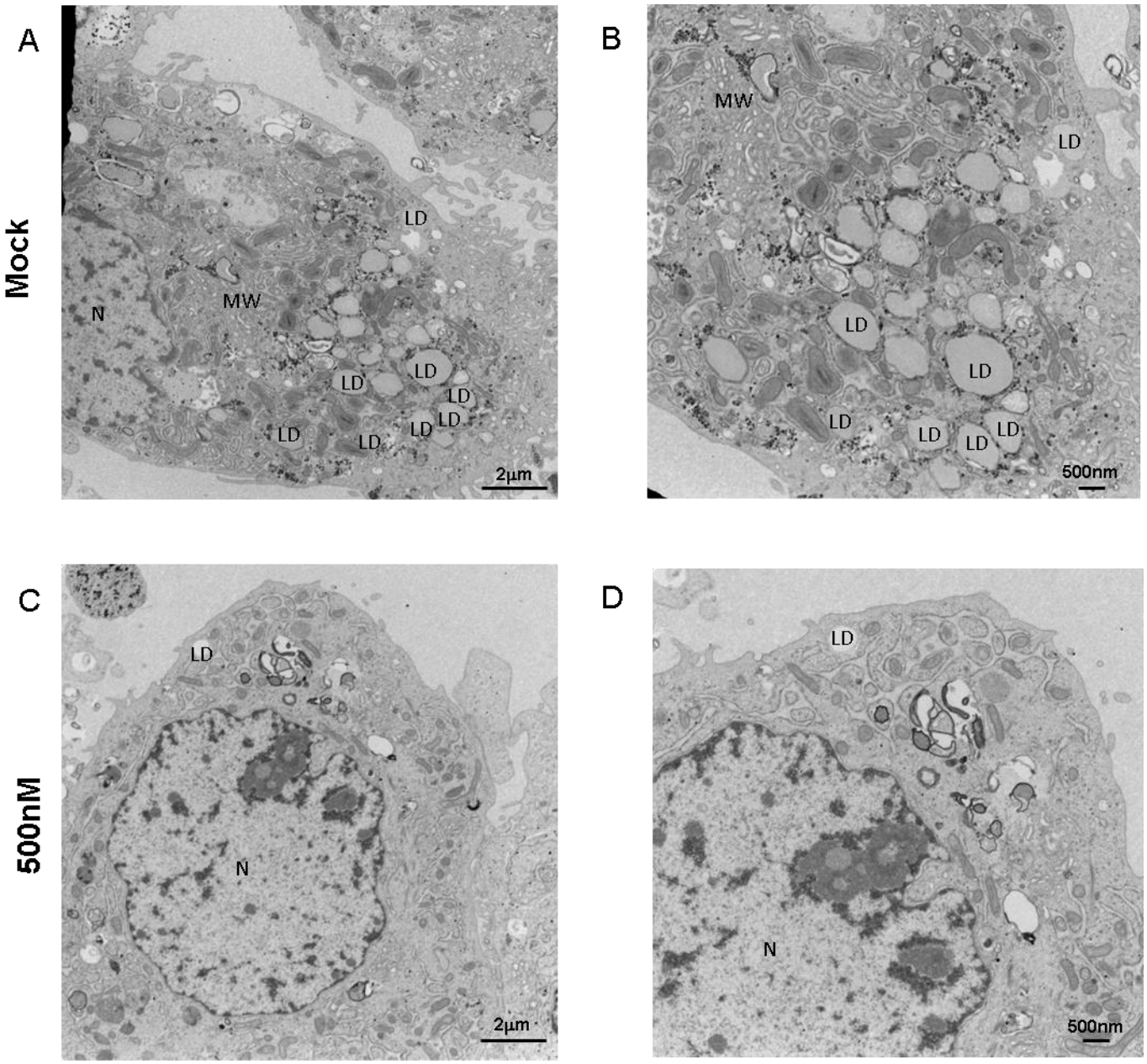


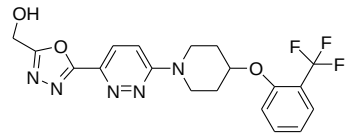
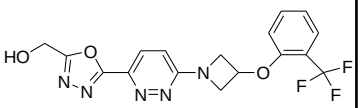
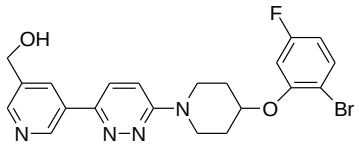
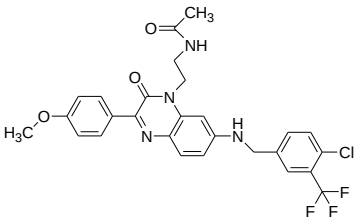
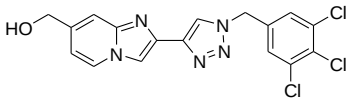
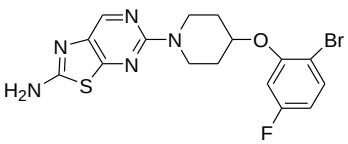

**Supplementary Figure S4: HCV RNA susceptibility to exogenously added nuclease after inhibiting SCD-1.** (**a**) Huh7-SGR cells were treated with vehicle (DMSO) (Lanes 1-6) or a concentration of inhibitor **A** yielding a 50% reduction in HCV RNA levels (Lanes 7-11) for 96 hours. RT-PCR was performed to illustrate the relative differences in HCV RNA abundance (n=4). The second trial (N2) was shown as a representative image in **Figure 2**. (**b**) The densitometry for each trial in (**a**) is quantified and individually shown. (**c**) The change in HCV RNA abundance between digitonin and digitonin + nuclease treatments for both mock and inhibitor **A** treated cells is shown. This graph demonstrates that SCD-1 inhibition with inhibitor **A** increases susceptibility of HCV RNA to exogenously added nucleases in digitonin permeabilized cells.

# Supplementary Figure S5

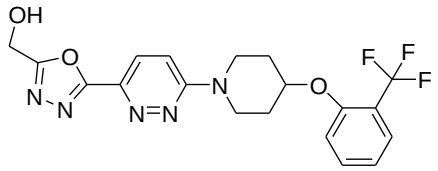
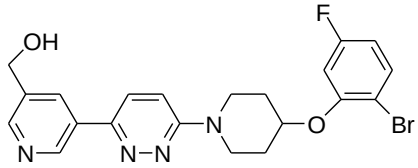
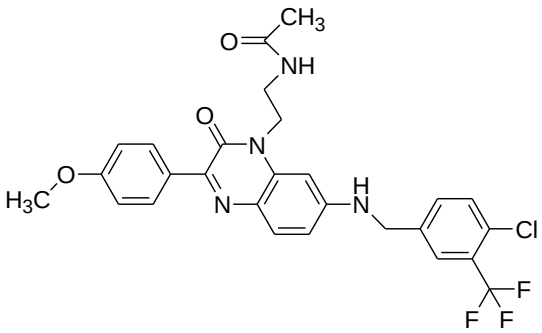
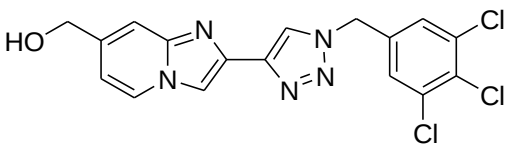
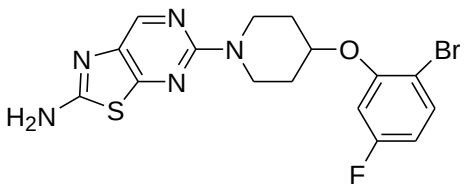



**Supplementary Figure S5: Electron microscopy shows that high dose treatment with inhibitor A reduces the appearance of membranous webs and the size and number of lipid droplets in Huh7-SGR cells.** Huh7-SGR cells treated for 96h with vehicle (DMSO) (**a**-**b**) or 500nM inhibitor A (**c**-**d**) were fixed and processed for EM. One representative cell is shown for each treatment at magnifications of 1500X (**a**,**c**) and 2500X (**b**,**d**) MW: membranous webs, LD: lipid droplets, N: nucleus

**Supplementary Table S1:** Palmitoleic acid or oleic acid treatment rescues SCD inhibitors' antiviral effect.

| Inhibitor | Structure | Inhibition | | | Rescue | | |
|---|---|---|---|---|---|---|---|
| | | $EC_{50}$ (nM) | | | | | |
| | | Gt1b | Gt1a | Gt1b 50%NHS | 10% FBS | 100 µM Oleic acid | 100 µM Palmitoleic acid |
| C |  | 1.9 | 0.74 | >40000 | 0.64 | 6382 | 3442 |
| D |  | 0.9 | 2.2 | >40000 | | | |
| E |  | 0.32 | 0.90 | >40000 | | | |
| F |  | 12 | 5.8 | >40000 | 6.4 | 14213 | 10941 |
| G |  | 13 | 25 | >40000 | 16 | >40000 | >40000 |
| H |  | 8.3 | 10 | >40000 | | | |

**Supplementary Table S2:** SCD inhibitors' do not directly repress HCV NS3 protease and NS5B polymerase activity *in vitro*.

| Inhibitor | Structure | NS3 $EC_{50}$ (nM) | NS5B $EC_{50}$ (nM) |
|---|---|---|---|
| C |  | >100000 | 30064 |
| E |  | >100000 | >80000 |
| F |  | >100000 | |
| G |  | >100000 | 32423 |
| H |  | >100000 | >80000 |

**Supplementary Table S3:** List of primers

| Primer Name | Nucleotide Sequence |
|---|---|
| 18S rRNA FWD | GCGATGCGGCGGCGTTATTC |
| 18S rRNA REV | CAATCTGTCAATCCTGTCCGTGTCC |
| HCV IRES FWD | GTCTGCGGAACCGGTGAGTA |
| HCV IRES REV | GCCCAAATCTCCAGGCATT |
| JFH1 HCV IRES REV | GCCCAAATGGCCGGGCATA |