SUPPLEMENTARY DIGITAL CONTENT

SUPPLEMENTARY MATERIALS AND METHODS

Plasmids, Viruses and Cells.

A plasmid containing the full-length complementary DNA of a genotype 2a Japanese Fulminant Hepatitis 1 (JFH-1) HCV isolate with three cell culture-adapted mutations that increase overall viral replication (JFH-1_T) and a replication-defective GNN plasmid (containing the indicated mutations in the viral polymerase active site GDD motif) were provided by R.S. Russell (Memorial University)[1]. Human hepatoma (Huh-7.5) cells were kindly provided by C.M. Rice (Rockefellar University) and were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (Wisent) and 10 mM nonessential amino acids.

In vitro Transcription, Transfection, and Production of Cell Culture-Derived HCV.

Infectious HCV particles were produced as described previously[1]. Briefly, the JFH-1_T and JFH-1_T GNN plasmid DNA were linearized, *in vitro* transcribed into viral RNA, and subsequently transfected into naïve Huh-7.5 cells. Infectious supernatants were collected at 5 days post-transfection, and after two passages through Huh-7.5 cells, viral stocks were concentrated using an Amicon Ultra-15 Filter (Millipore). Viral titers were determined by endpoint dilution assay using Focus Forming Units (FFU).

Indirect Immunofluorescence.

For indirect immunofluorescence analysis, cells were plated into 8-well chamber slides at 2 days post-infection and incubated for an additional 3 days to allow foci to form. For intracellular staining, slides were washed with 1X Phosphate Buffered Saline (PBS) and fixed with acetone for 2 min at room temperature. Mouse monoclonal anti-HCV core antibody (Anogen Core B2 Antibody) was used at a dilution of 1:200 followed by incubation with a 1:400 dilution of Alexafluor 488 goat anti-mouse antibody (Bio-Rad). Finally, slides were counterstained with DAPI (Vectashield Mounting Medium with DAPI, Vector Laboratories). Immunofluorescence images were taken using an Axiovert (Zeiss) microscope at x10 and x20 magnification. We used a conservative estimate for quantification of the FFU assay whereby wells were considered positive if they contained three or more HCV-core positive foci (with 8 wells plated per contaminated vial).

RNA Quantification by Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR).

Total RNA was isolated from Huh-7.5 cells using TRIzol reagent (Thermo Fisher Scientific) according to the manufacturer's instructions and quantified using a Nanodrop spectrophotometer. Quantitative RT-PCR reactions were performed using iTaq Universal Probe Kit (BioRad) with 500 ng of total RNA using the following primers: forward primer, 5′-GCC ATG GCG TTA GTA TGA GTG T-3′; reverse primer, 5′-CGC CCT ATC AGG CAG TAC CAC AA-3′; probe, 5′-6-FAM-TCT GCG GAA CCG GTG AGT ACA CC -3′. *In vitro* transcribed JFH-1_T HCV RNA was used for calibration curves and absolute quantification.

Cell Viability Assay.
Huh-7.5 cells were seeded at 1 x 10^4 cells/well in 96-well plates (Corning). The following day, serial dilutions (2-fold) of Dexamethasone, Lidocaine, Neostigmine, Phenylephrine, Propofol, Rocuronium and vehicle (saline) were prepared in cell culture media and incubated with Huh-7.5 cells. After a 5 hour (h) incubation, the medications were removed and replaced with fresh cell culture media. Cell viability was assessed via alamarBlue assay (Invitrogen) as per the manufacturer's instructions.

References

1. Russell RS, Meunier JC, Takikawa S, et al. Advantages of a single-cycle production assay to study cell culture-adaptive mutations of hepatitis C virus. *Proc Natl Acad Sci U S A*. 2008;105(11):4370-4375.

SUPPLEMENTARY TABLES

Table S1. Medication concentrations that maintain 90% cell viability.

Medication	Concentration	Concentration >90% Cell Viability (95% Confidence Interval)
Dexamethasone	4 mg/mL	0.895 μM (0.835 to 0.959)
Lidocaine	10 mg/mL	0.603 μM (0.567 to 0.641)
Neostigmine	10 mg/mL	2.064 μM (1.966 to 2.166)
Propofol	10 mg/mL	7.345 μM (7.059 to 7.644)
Rocuronium	10 mg/mL	1.372 μM (1.315 to 1.431)
Phenylephrine	1 mg/mL	0.049 μM (0.019 to 0.126)

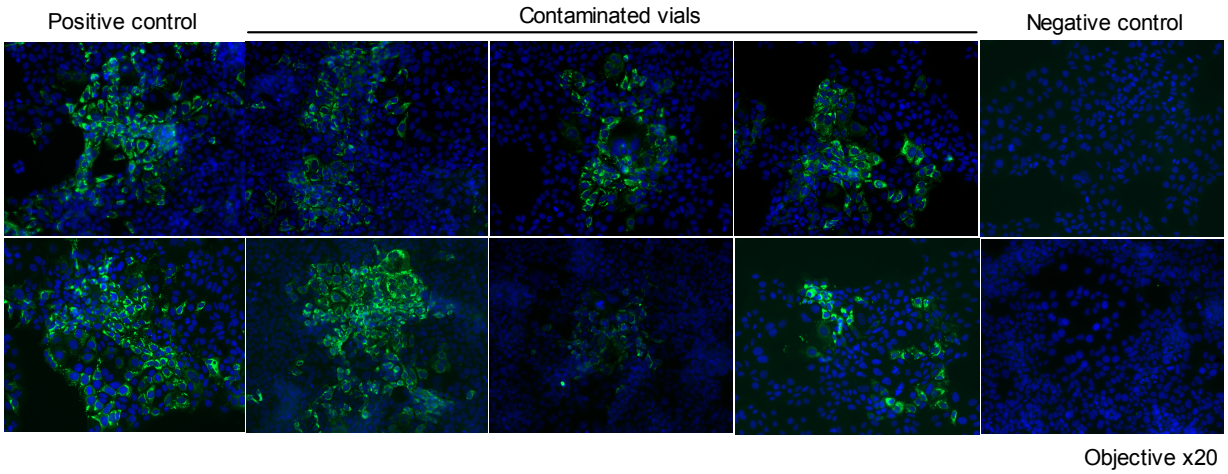

Figure S1. HCV foci are readily observed after transfer via sterile needle and syringe into medication vials when the rubber diaphragm is contaminated at all HCV titers tested. Representative fluorescence microscopy images from 8-well chamber slides grown with cell culture media from medication vials contaminated with intermediate (2.56×10^6 IU/mL, top) and low titer (8×10^5 IU/mL, bottom) HCV. Cells were fixed and stained with DAPI (blue, cell nuclei) and anti-HCV core antibody (green, HCV core protein). Images are representative of at least three independent experiments.

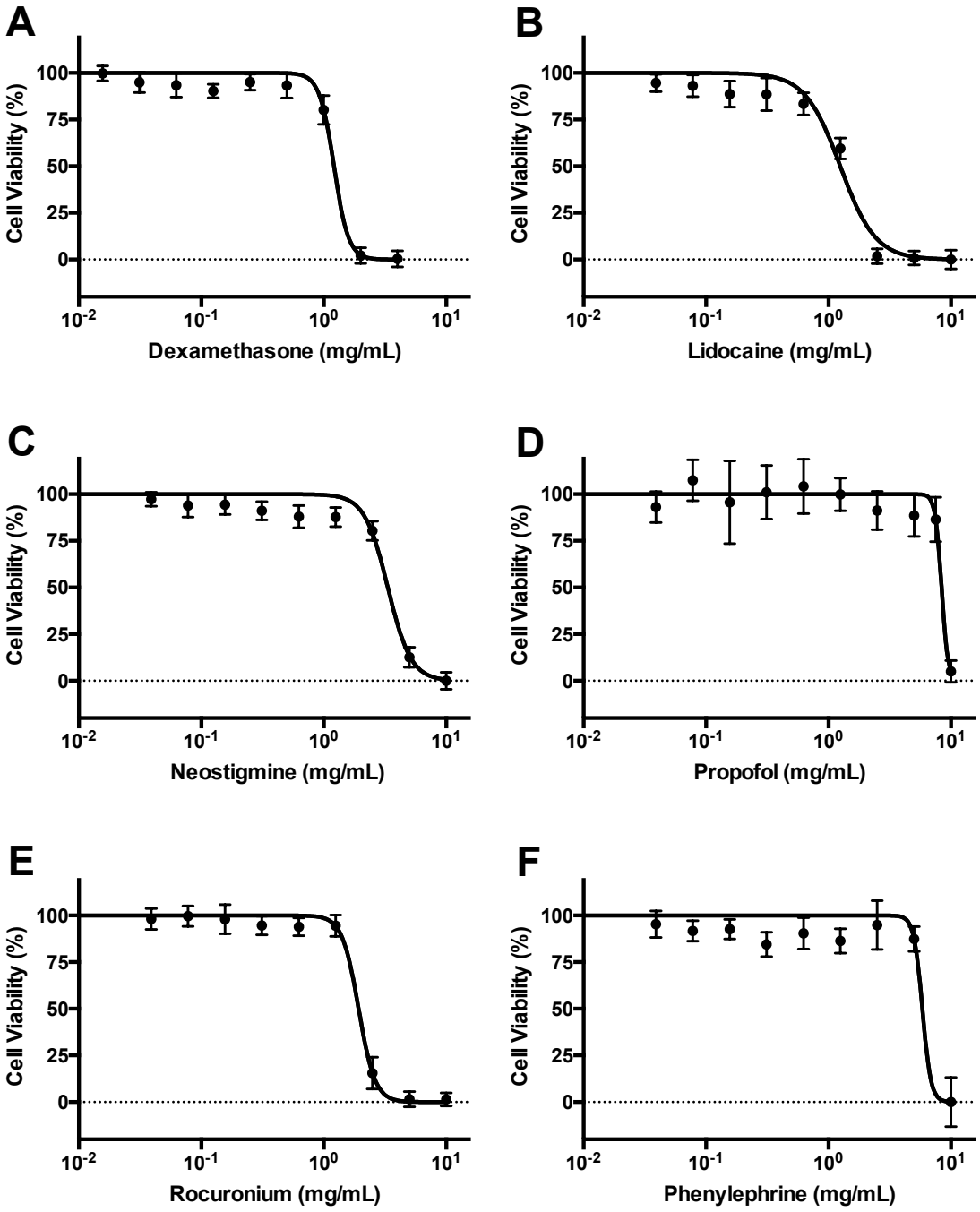

Figure S2. Cell viability dose-response curves of medications in Huh-7.5 cells. Cell viability was determined at 5 h post-treatment in Huh-7.5 cells using alamarBlue for A) Dexamethasone, B) Lidocaine, C) Neostigmine, D) Phenylephrine, E) Propofol, and F) Rocuronium. All data are representative of at least three independent experiments and error bars represent standard deviation.