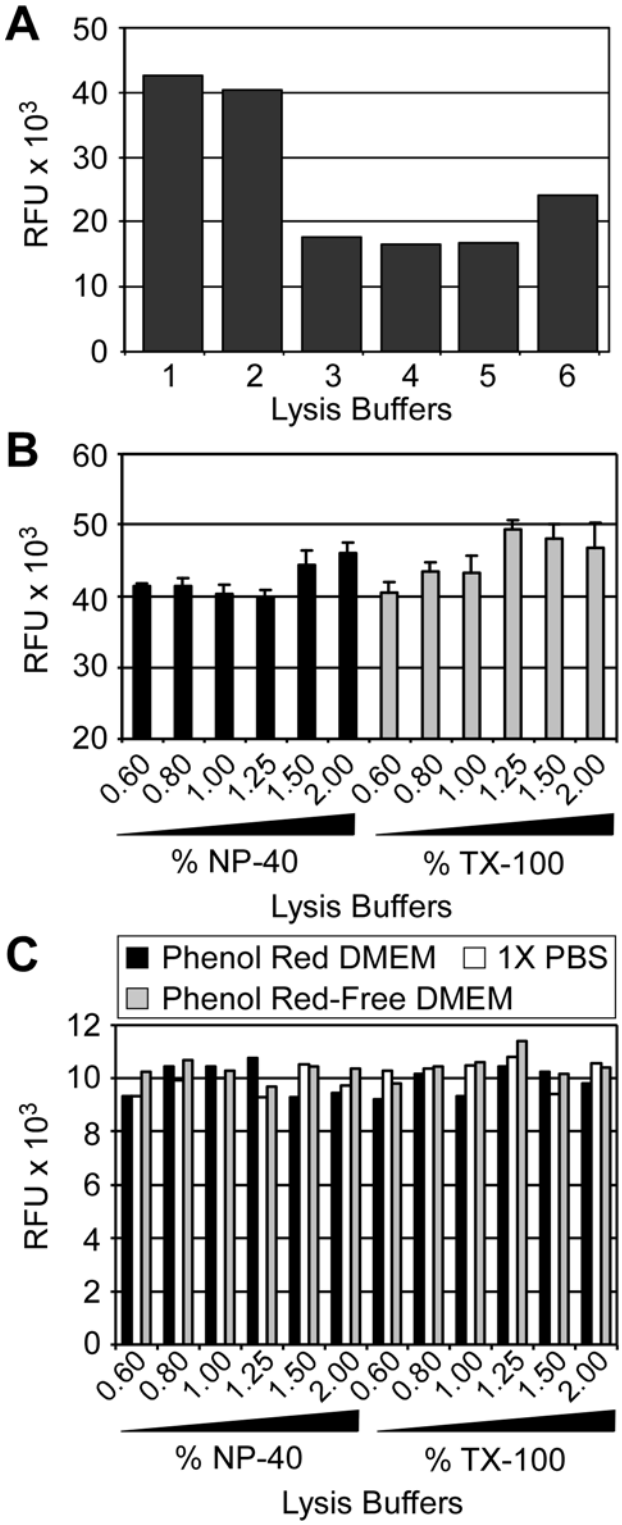
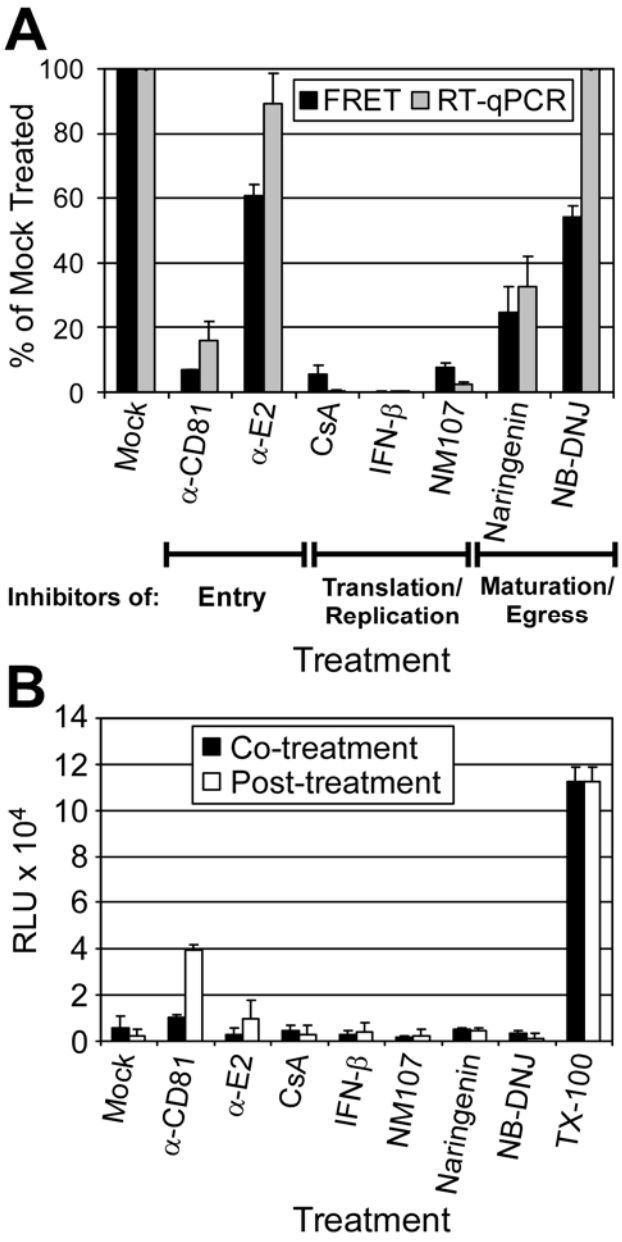
SUPPLEMENTARY FIG. 1.



SUPP FIG. 1. Lysis conditions for cell-based FRET assay. (A) To determine optimal lysis buffer for the NS3 FRET assay, Huh7 cells were cultured in 1% DMSO DMEM medium for 20 days. At day 20, cells were inoculated with HCVcc JFH-1 at an MOI of 0.01 FFU/cell and infected for 10 days. On day 10 p.i., cultures were lysed with 50 μl (1) 1.0% Triton -100 lysis buffer (Triton X-100, 50mM Tris-HCl, pH7.5, 150mM NaCl, 2mM EDTA), (2) 1.0% NP-40 lysis buffer (NP-40, 50mM Tris-HCl, pH7.5, 150mM NaCl, 2mM EDTA) (3) 1% CHAPS buffer (CHAPS, 50mM Tris-HCl, pH7.5, 150mM NaCl, 30mM DTT) (4) RIPA buffer (1%Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 50mM Tris-HCl, pH7.5, 150mM NaCl, 2mM EDTA) (5) 1.0% sodium deoxycholate buffer (sodium deoxycholate, 50mM Tris-HCl, pH7.5, 150mM NaCl, 2mM EDTA) or (6) 1X Anaspec Lysis Buffer and NS3 protease activity was monitored by FRET. (B) To determine the optimal detergent concentration, infected cultures were lysed at day 10 p.i. with 50 μl of 0.6, 0.8, 1, 1.25 1.50 or 2.00% NP-40 lysis buffer or 0.6, 0.8, 1, 1.25 1.50 or 2.00% Triton X-100 lysis buffer and NS3 protease activity was monitored by FRET. (C) To determine the affect of phenol-red on FRET activity, infected cultures were incubated in complete DMEM or phenol-red free complete DMEM. Day 10 p.i., medium was removed, one set of triplicate samples were washed once with 100 μl of 1X PBS, samples were lysed with 50 μl of 0.6, 0.8, 1, 1.25 1.50 or 2.00% Triton X-100 lysis buffer or 0.6, 0.8, 1, 1.25 1.50 or 2.00% NP-40 lysis buffer and NS3 protease activity was monitored by FRET. Shown are RFU minus HCV-negative background detected at cycle 20.

SUPPLEMENTARY FIG. 2.



SUPP FIG. 2. Identification of inhibitors that act throughout the HCV lifecycle. (A) DMSO-Huh7 cells were infected with HCV at 0.05 FFU/cell and treated post-infection on days 2 and 4 p.i. with HCV inhibitors that act a different stages of HCV infection: 50 μg/ml α-CD81, 100 μg/ml α-E2, 2.5 μM CsA, 250 U/ml IFN-β, 18.5 μM NM107, 200 μM Naringenin, and 500 μM NB-DNJ. Day 6 p.i., triplicate cultures were assayed for HCV RNA levels by

RTqPCR and NS3 protein levels by FRET. Data are presented as a percentage of mock-treated. (B) Inhibitor-induced toxicity was determined on day 6 p.i. after inhibitor co-treatment (Fig. 6) or post-treatment (Supplementary Fig. 2A) by measuring the release of adenlyate kinase by the Huh7 cell cultures using the ToxiLight® Non-destructive Cytotoxicity luminescence assay kit. Triton X-100 (TX-100) lysed Huh7 cells served as positive control for maximum AK release from DMSO-Huh7 cells.