Fig. S1. Schematic of effects of HCV NS5A on Kv2.1-mediated apoptosis. Phosphorylation and activation of p38 MAPK is a response to oxidant-induced stress (e.g., DTDP treatment) (1). Activated p38 MAPK then phosphorylates the Kv2.1 channel at S800 (2), facilitating insertion of Kv2.1 channels into the plasma membrane (3). The concomitant increase in K\(^+\) efflux results in a reduction in intracellular K\(^+\) (4), activating caspases (5), and inducing apoptosis (6). During HCV infection, the NS5A protein prevents p38 MAPK phosphorylation in a polyproline motif-dependent fashion (7). This blocks p38 MAPK-dependent phosphorylation of the Kv2.1 channel at S800 (2), thereby preventing membrane insertion of additional Kv2.1 channels (3). By inhibiting this pathway at the level of p38 activation, NS5A prevents subsequent cellular events and thereby provides a means for virus survival. Solid lines indicate active pathways, dashed lines indicate inactive pathways.
Fig. S2. Kv2.1 function is specifically perturbed in cells containing the HCV replicon. (a) RepliconWT and Kv2.1 expression in the HEK293 Kv2.1 cell line was confirmed by immunofluorescence for NS5A (green) and Kv2.1 (red). (b) All Huh-7 cell lines display similar Kv2.1 levels. Western blots showing the Huh-7 Kv2.1 band that runs at ~90 kDa compared with the HEK293 Kv2.1 lysate that exhibits a diffuse band near 100 kDa. The differences in apparent molecular mass is most likely because of differential patterns of phosphorylation on sites other than S800 (12). (c) All Huh-7 cell lines tested display similar Kv2.1 distribution. Cells were fixed with methanol, permeabilized, and probed with sheep anti-NS5A antibodies and anti-sheep Alexa Fluor 488 secondary antibodies. Cells were co-labeled with mouse anti-Kv2.1 followed by staining with anti-mouse Alexa Fluor 594 to show Kv2.1 expression. Representative confocal images are shown. Images on the Right are higher magnifications of the boxed areas. (d) Nuclear fragmentation induced by DTDP is perturbed in repliconWT cells through Kv2.1 inhibition. Cells from the experiment in Fig. 4A were fixed, stained, and mounted as described. Representative images are shown. Nuclei (blue) that showed fragmentation or abnormal shape were deemed as apoptotic. NS5A staining is green.
Fig. S3. Kv2.1 is expressed in primary, untransformed human hepatocytes. Primary human hepatocytes were analyzed by (a) immunofluorescence or (b) Western blot with an anti-Kv2.1 antibody as described in the Materials and Methods. The Right hand lanes of the western blots show lysates from Huh-7 cells, equal protein loading of the two lysates in (b) was verified with an anti-GAPDH blot. (c) Primary human hepatocytes were analyzed by whole-cell patch clamp either before (control) or after application of 100 μM DTDP for 10 min. Representative traces of outward K⁺ currents evoked by step depolarizations are shown. (d) I/V relationships for: Primary human hepatocytes in the absence (■) or presence (▲) of 5 mM TEA (n = 6–8 cells). Insets show representative traces of outward K⁺ currents evoked by step depolarizations. [Scale bars for Inset, 20 pA (vertical) and 50 ms (horizontal).]