Supplementary Figure Legends

sFigure 1

Huh7.5 cells were incubated with a serial dilution of RBV for 72hrs in triplicate. AlamarBlue reagent was added for 3hrs and Fluorescence intensity was assessed as a measure of cytotoxicity.

sFigure 2

(A) HCV genotype 1-7 amino acid sequences were aligned and the table shows the amino acid at the positions of interest and compared to the gt3a reference sequence. (B) Alignment of 1200 HCV genotype 3a sequences from the HCV-GLUE database identifying the minor variants at each position of interest. Legend designates the colour code for each amino acid. (C) Analysis of the NS5b polymorphisms in the BOSON cohort, the prevalence of the NS5b polymorphisms was assessed from >500 viral sequencing samples obtained from the BOSON cohort. (D) Each mutation was tested to assess whether the frequency was significantly different between SVR and non-SVR samples. Frequencies were analysed using a Fisher's exact test.

sFigure 3

Huh7.5-SEC cells transiently transfected (by electroporation) with luciferase containing HCV (S52-replicon) constructs with the indicated NS5b polymorphisms were assessed. Cells were treated with RBV for 72hrs and replication was measured using a luciferase assay, normalised to a sample 4 hours post electroporation for each construct. Panels A-E indicate the effect of the polymorphisms from patient 9 to RBV sensitivity. The above is representative of at least 3 independent experiments.

sFigure 4

Huh7.5-SEC cells transiently transfected (by electroporation) with luciferase containing HCV (S52-replicon) constructs with the indicated NS5b polymorphisms were assessed. Cells were treated with RBV for 72hrs and replication was measured in a luciferase assay, normalised to a sample 4 hours post electroporation for each construct. Panels A-C indicate the effect of the polymorphisms from patient 10-14 on RBV sensitivity. The above is representative of at least 3 independent experiments.

sFigure 5

Huh7.5-SEC14L2 cells transfected with the viral constructs were stained for the HCV NS5a protein (green) by immunofluorescence 7 days post-transfection to confirm

establishment of replication complexes. Mock transfected cells were included as a negative control.

sFigure 6

(A) Naïve Huh7.5-SEC14L2 cells were infected with Wt and DBN virus with the polymorphisms of interest at a MOI of 0.05. Samples were taken at the indicated time points for quantification of HCV RNA by RT-qPCR. Data presented is representative of 2 independent experiments and analysed using a Mann-Whitney U-Test. Graph depicts mean ±SEM. (B-C) An equal inoculum of the wt and mutant virus was used to infect the cells (MOI = 0.03 $TCID_{50}$), the relative amounts of the competing viruses were determined by NGS at 0, 24, 48 and 72hrs post infection and the % of wt (alanine at 150 or lysine at 206) is shown.

sTable 1

Details of the samples used in the capture fusion assay (figure 1 and table 1). Data includes treatment, clinical outcome, age, gender, disease status and baseline viral load.

sTable 2

The calculated IC_{50} values with 95% confidence intervals and the fold change compared to the WT replicon from sFig 3-4 were replicons were treated with RBV, is summarised. Relative luciferase levels compared to the WT replicon construct for each polymorphism were also included.