Supplemental Materials and Methods

**Identification of NS5B sequences for chimeric replicons.** Full length NS5B sequences from genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a, and 6 were downloaded separately by genotype from the European HCV database and then aligned using the program MUSCLE (1). For genotypes 1a and 1b, the frequency of each residue in each column was calculated. Maximum likelihood phylogenetic trees were generated of the alignments. 9-10 clinical sequences were identified as representative of the breadth of each subtype using a clustering program (2) that clusters sequences based on percentage identity and also identifies a representative sequence for each cluster. The percentage identity was adjusted until eight to ten clusters were generated. The representative patient sequences used to make the chimeric replicons are displayed on the phylogenetic tree (Figures S1 and S2). Representative sequences that did not result in viable chimeric replicons were replaced with a patient sequence from the same cluster. For genotypes 2-6, the consensus for each gene was generated using the Advanced Consensus Maker (http://www.hiv.lanl.gov/content/sequence/CONSENSUS/AdvCon.html). The parameters used were unanimous value = 1.00, majority value = 0.50, use most common character, break ties with IUPAC characters. Clinical isolates that were the closest to the consensus sequences were identified using Blast. No viable chimeric replicons could be obtained with genotype 6 sequences.

**Sequence analysis of resistant mutants.** 1 µg RNA was mixed with 3 µL HCV-RT primer (100 µM) and $H_2O$ to a final volume 12.5 µL. The mixture was incubated at 65°C for 10 min. 4 µL Transcriptor buffer (Roche), 2 µL dNTPs (1 mM), 1 µL RNAsin (Promega) and 0.5 µL Transcriptor reverse transcriptase (Roche) were then added to the mixture followed by 1 hr incubation at 55°C. For population sequencing, the NS5B gene was amplified from the cDNA using genotype specific primers. For genotype 1a the forward primer was 5'-TCAATGTCTTATTCCTGGACAG-3' and the reverse primer was 5'-TCATCGGTTGGGGAGGAGGTAG-3'. For genotype 1b the forward primer was 5'-TCGATGTCCTACACATGGACAG-3' and the reverse primer was 5'-TCATCGGTTGGGGAGTAGATAG-3'. PCR products (20 µL) were purified using Agencourt Ampure magnetic bead. Gene specific primers were designed in both sense and antisense directions using GSK PrimerD (GSK in-house developed) software to achieve double strand coverage. DNA sequencing reactions were set up using 40 ng of PCR product and analyzed on the Applied Biosystems 3730xl DNA Sequencer. Electropherograms were edited and assembled to HCV reference sequences using GeneCodes Sequencher (v4.9) software. For heterozygous mutation analysis, the sensitivity level for detecting secondary peaks is set between 15-20% of the major peak height. To remove false positives, follow-up analysis is performed visually for each nucleotide position at which a variation is indicated by the analysis software. False negatives are also alleviated by visual inspection of the sequence alignments. Mutations that are evident in both sense and antisense sequences are reported.

For 454 sequencing, tiled PCR primer sets with average lengths of 240 base pairs (standard 454 sequencing) or 450 base pairs (titanium sequencing) for NS5B for both 1a and 1b genotypes were designed using GSK Primer D software. Adaptor deoxyoligonucleotides of 18 bases were linked to the specific primers according to 454 Amplicon Sequencing instructions.

PCR reactions containing 50 ng total cDNA were carried out using Platinum Taq DNA polymerase along with standard amplification reagents. Amplicon samples for each sample were diluted and combined in equimolar ratios resulting in 200,000 molecules/µL of DNA per sample to create an amplicon library. Amplicon libraries prepared as described above were entered directly into the 454/Roche sequencing pipeline, with the only major variable being the amount of DNA from the pooled sample used to seed the emulsion PCR reactions. All subsequent procedures were carried out essentially as described in the GSFLX guide-book for amplicon sequencing (Roche Life Sciences, Indianapolis, IN Cat # 04932315001). Each sample was directly sequenced on one or two 16th regions of a full GSFLX Standard sequencing plate.

Supplementary References


1. Edgar R. C. 2004. MUSCLE:multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792-1797
2. Huang, Y., B. Niu, Y. Gao, L. Fu, and W. Li. 2010. CD-HIT Suite: a web server for clustering and comparing biological sequences. Bioinformatics 26:680-682

| Genotype | Accession Number |
|---|---|
| 1a | AB520610, EF407418, EF407457, EU781777, EU781802, EU781804, EU781807, EU862836, EU862840 |
| 1b | AB049090, EF407504, EU155306, EU155372, EU234062, EU256088, EU482881, AB154183, EU155381 |
| 4a | DQ418788 |
| 5a | AF064490 |

Table S1. Accession numbers of NS5B sequences in chimeric replicons

| Virus | % Reduction in CPE | % Cell Viability |
|---|---|---|
| Adenovirus Type 1 | 6.9 | 100.0 |
| Adenovirus Type 3 | 22.4 | 100.0 |
| Adenovirus Type 40 | 3.5 | 95.3 |
| BVDV | 0.8 | 100.0 |
| Coxsackie A7 Virus | 2.0 | 99.7 |
| Coxsackie B4 Virus | 0.0 | 100.0 |
| Dengue Virus Strain 1 | 0.0 | 100.0 |
| Dengue Virus Strain 2 | 16.6 | 100.0 |
| Dengue Virus Strain 3 | 0.8 | 98.5 |
| Dengue Virus Strain 4 | 8.3 | 95.4 |
| Enterovirus | 4.1 | 100.0 |
| HSV-1 | 20.2 | 100.0 |
| HSV-2 | 6.9 | 100.0 |
| Influenza A Strain | 9.2 | 100.0 |
| Influenza B Strain | 1.3 | 100.0 |
| Measles Virus | 3.5 | 100.0 |
| Parainfluenza Virus | 8.6 | 97.5 |
| Poliovirus | 14.5 | 100.0 |
| RSV | 4.0 | 100.0 |
| Rhinovirus | 2.2 | 96.3 |
| Rotavirus | 4.5 | 98.5 |
| Yellow Fever Virus | 0.1 | 98.8 |

Table S2. In vitro antiviral results for 10 μM GSK2485852. Antiviral activity was determined by the ability to protect the cells from the cytopathic effects (CPE) of viral infection as measured by cell viability using the MTS assay. The cytotoxicity of 10 μM GSK2485852 was determined on uninfected cells using the MTS assay.

| DAA combined with GSK5852 | Combination Index | Results |
|---|---|---|
| GSK5852 | 0.92 | Nearly Additive |
| NS3 Protease Inhibitor (BILN2061) | 1.01 | Nearly Additive |
| NS4B Inhibitor (GSK5337A) | 0.86 | Slight Synergy |
| NS5A Inhibitor (GSK6805) | 0.97 | Nearly Additive |
| NS5B Nucleoside (2'-C-methylcytidine) | 0.94 | Nearly Additive |
| NS5B NNI thumb pocket 1 (Merck NNI) | 0.87 | Slight Synergy |
| NS5B NNI thumb pocket 2 (filibuvir) | 1.06 | Nearly Additive |
| NS5B NNI palm site 1 (SB-711845) | 1.06 | Nearly Additive |
| Cyclosporine A | 1.08 | Nearly Additive |
| Interferon α | 0.82 | Slight Synergy |
| Ribavirin | 0.99 | Nearly Additive |

Table S3. The Combination Index (CI) of GSK5252 in combination with other antivirals. The interpretations of the CI value are based on recommendations provided by CalcuSyn.

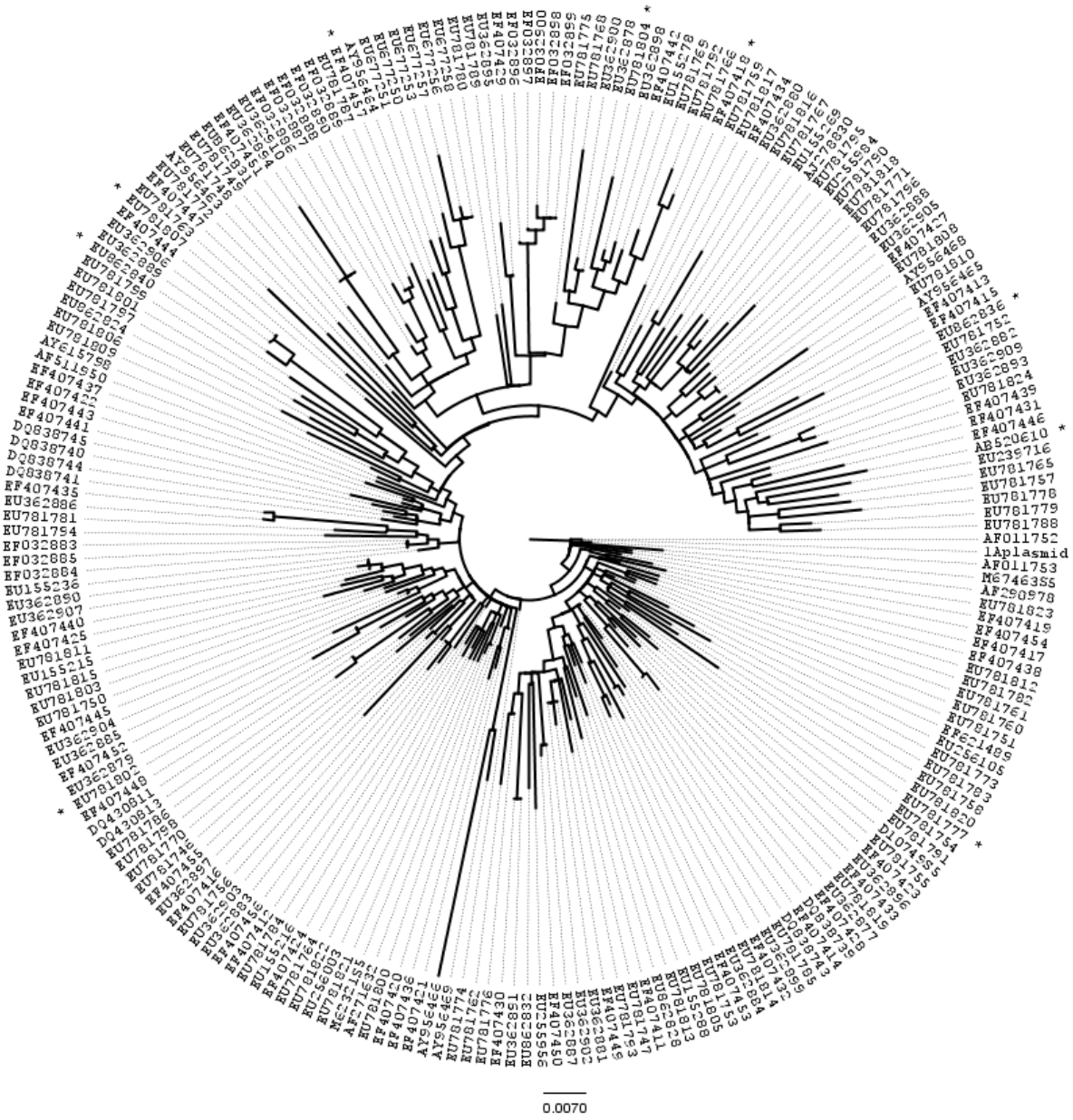


**Figure S1. Phylogenetic tree of genotype 1a NS5B sequences**. Accession numbers of isolates used to make NS5B chimeric replicons are indicated with an asterisk.

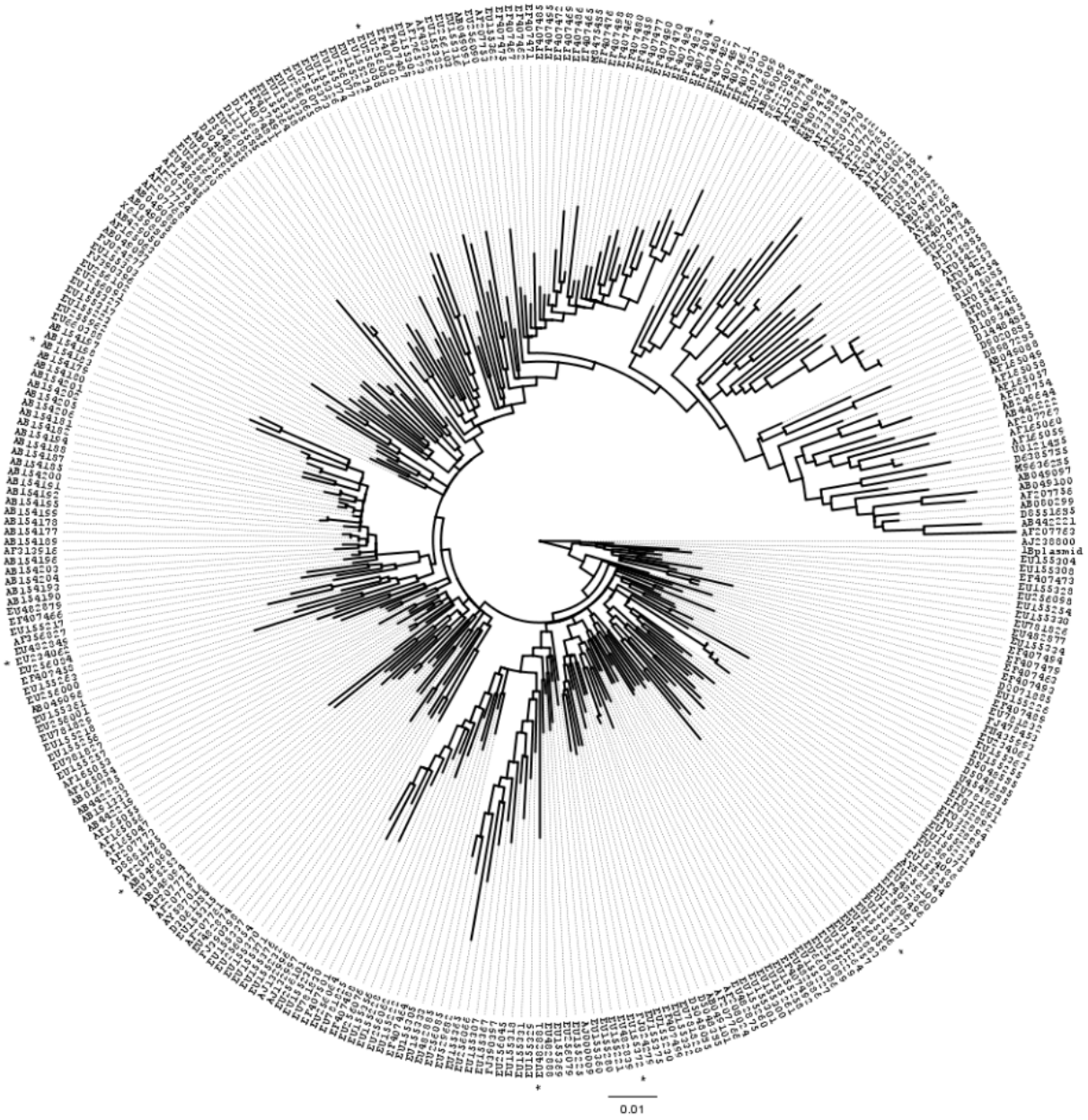


**Figure S2. Phylogenetic tree of genotype 1b NS5B sequences**. Accession numbers of isolates used to make NS5B chimeric replicons are indicated with an asterisk.