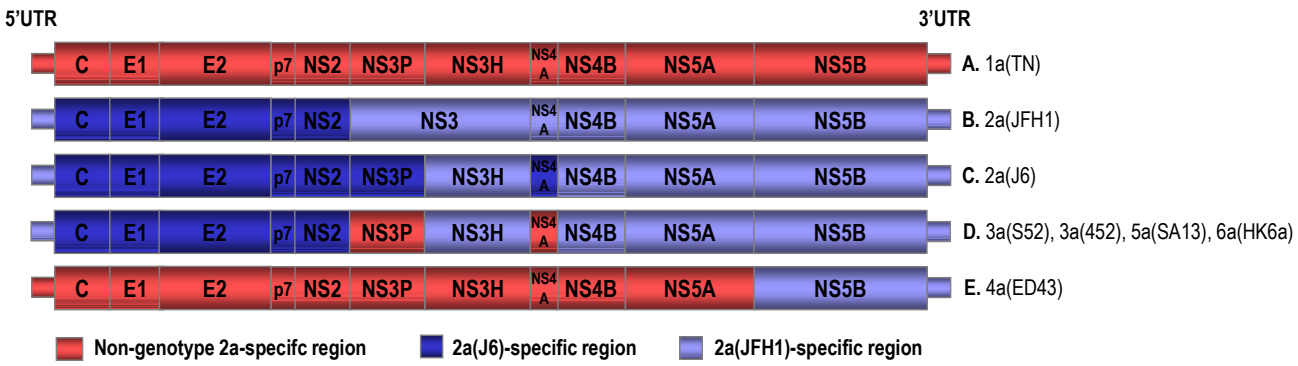
Supplementary Materials



Supplementary Figure S1. Graphical presentation of HCV recombinants used in this study. The recombinants used were designated according to the genotype(isolate) of their NS3P/NS4A sequence as indicated on the right. HCV proteins are indicated as C, Core; E1 and E2, envelope proteins 1 and 2; p7; NS2-NS5B, non-structural proteins 2-5B. 5´and 3´untranslated regions (UTRs) are indicated above the genomes. Genomic regions derived from genotype(isolate) 1a(TN), 3a(S52), 3a(452), 5a(SA13), 6a(HK6a), or 4a(ED43) are shown in red. Genomic regions derived from genotype(isolate) 2a(J6) or 2a(JFH1) are indicated in dark blue or light blue, respectively. (*A*) 1a(TN) is identical with TN full-length +LSGF/A1226G/Q1773H (1); for single-cycle production assays, a further adapted 1a(TN) full-length recombinant (TNcc) was used (1). *(B)* 2a(JFH1) is identical with J6/JFH1 (2) and contains Core-NS2 of genotype(isolate) 2a(J6) as well as 5´UTR and NS3-3´UTR of 2a(JFH1). (*C*) 2a(J6) is identical with 2a(J6) described in (3) and contains 2a(J6) specific Core-NS3P and NS4A, while remaining genome regions are from 2a(JFH1). (*D*) 3a(S52), 3a(452), 5a(SA13), and 6a(HK6a) are identical with 3a(S52)mut7, 3a(452), 5a(SA13)mut7, and 6a(HK6a)mut6, described in (3) and contain genotype(isolate)-specific NS3P/NS4A, while remaining genome regions are derived from 2a(J6) or 2a(JFH1) as indicated. (*E*) 4a(ED43) is identical with 4a(ED43)5-5A_LS/R781W/A1309P/A1786V (4) and contains 5´UTR-NS5A of genotype(isolate) 4a(ED43) and NS5B-3´UTR of 2a(JFH1).

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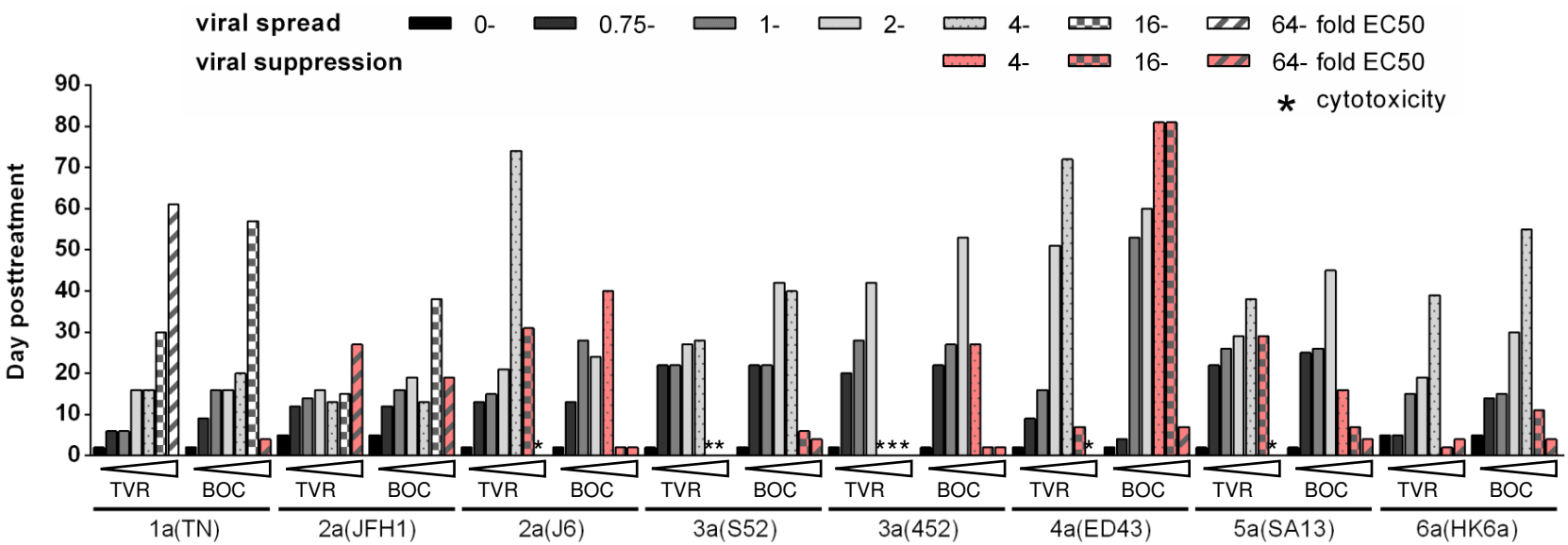
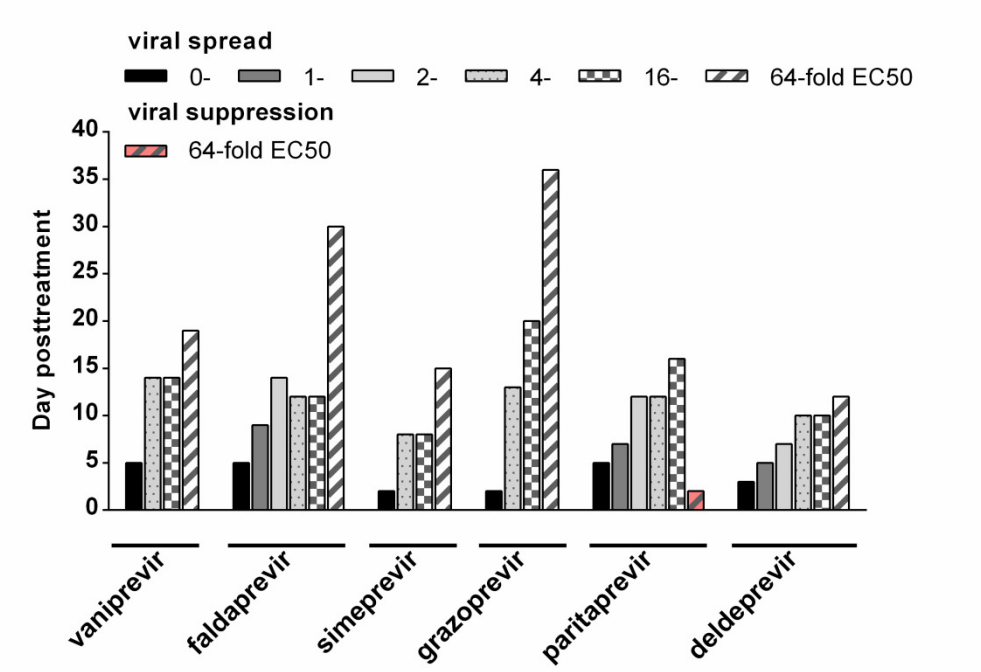
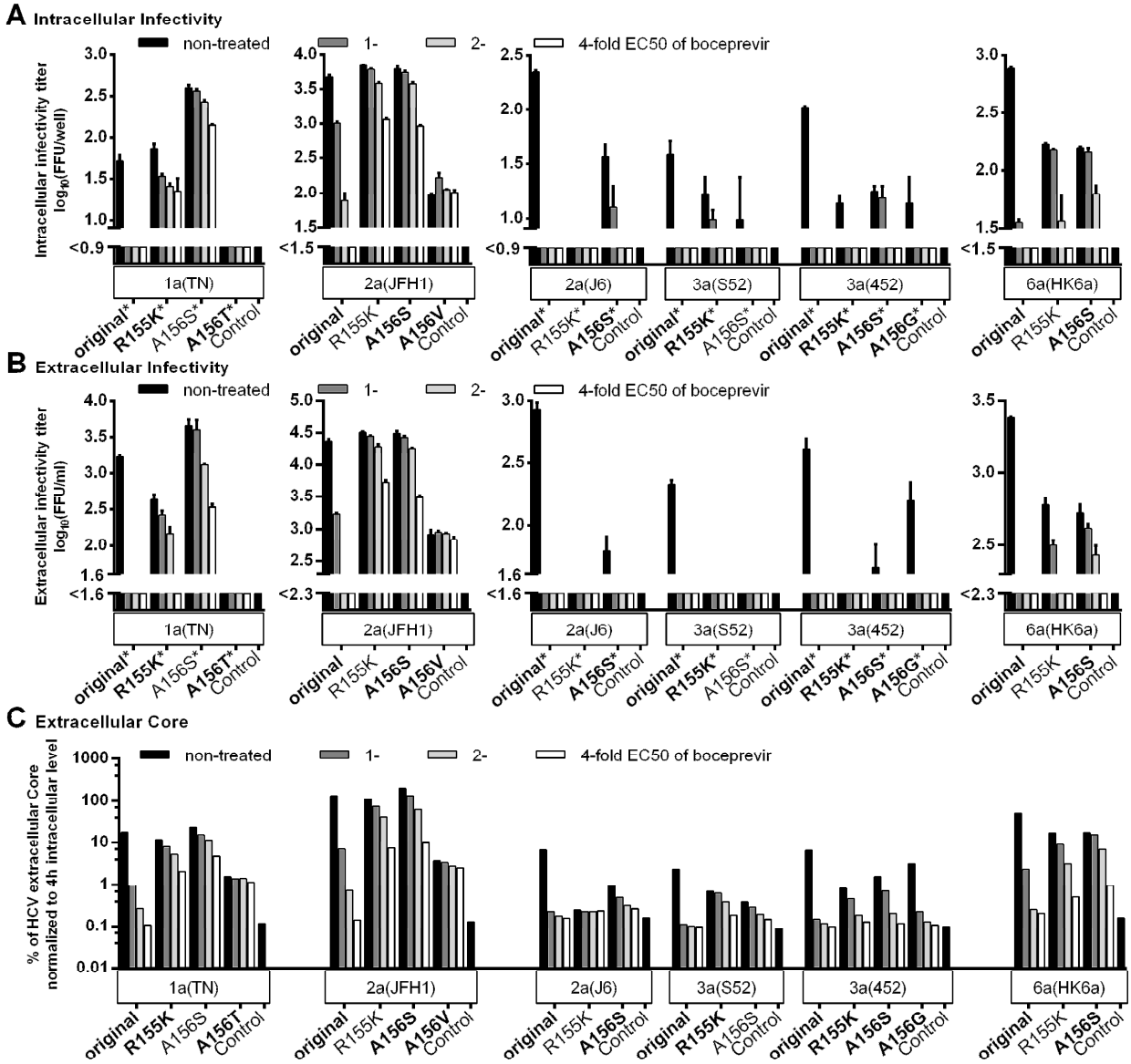

viral spread
0-
0.75-
1-
2-
4-
16-
64- fold EC50
viral suppression
4-
16-
64- fold EC50
* cytotoxicity
Day posttreatment
90
80
70
60
50
40
30
20
10
0
TVR
BOC
1a(TN)
2a(JFH1)
2a(J6)
3a(S52)
3a(452)
4a(ED43)
5a(SA13)
6a(HK6a)

Supplementary Figure S2. Induction of viral escape of HCV genotype 1-6 culture viruses treated with telaprevir and boceprevir. Huh7.5 cells were infected with recombinant viruses with the NS3P/NS4A of genotype(isolate): 1a(TN) (1), 2a(JFH1) (2), 2a(J6) (3), 3a(S52) (3), 3a(452) (3), 4a(ED43) (4), 5a(SA13) (3), and 6a(HK6A) (3). Treatment with either telaprevir (TVR) or boceprevir (BOC) was initiated when ~10-60% of culture cells were infected, as estimated by HCV-immunostaining. Cell cultures were treated three times per week, when cells were split, with 0.75-, 1-, 2-, 4-, 16-, or 64-fold EC50. The following specific EC50 (nM) values, determined in 48 h high throughput treatment assays (3,5), were used to calculate treatment concentrations for telaprevir and boceprevir, respectively: 109 and 128 for 1a(TN), 516 and 623 for 2a(JFH1), 844 and 1094 for 2a(J6), 3367 and 1435 for 3a(S52), 10393 and 2151 for 3a(452), 2159 and 1387 for 4a(ED43), 1202 and 933 for 5a(SA13), and 454 and 679 for 6a(HK6a) (1,3,4) (unpublished). Non-treated control cultures were followed until viruses spread to ≥80% of culture cells. Treated cultures were followed until the peak in the percentage of HCV-positive culture cells was observed (typically ≥50%), potentially representing viral escape, or until no HCV-positive cells were identified in six consecutive immunostainings, defined as viral suppression. For cultures showing viral spread, the first day following initiation of treatment with ≥80% (non-treated cultures) or ≥50% (treated cultures) HCV-positive cells is reported. For 2a(JFH1) and 3a(452) cultured with 2-fold EC50 of boceprevir, and 6a(HK6a) cultured with 0.75-fold EC50 of telaprevir, viral spread to only 40% of culture cells was observed and was followed by a decrease in the percentage of HCV-positive cells. For cultures showing viral suppression, the first day following initiation of treatment with no HCV-positive cells is reported. The culture infected with 4a(ED43) treated with 4-fold EC50 of boceprevir was stopped at day 80 following initiation of treatment despite the presence of few HCV-positive cells. Data shown in this graph were not necessarily obtained from the same experiment. *, fold EC50 of telaprevir not tested due to cytotoxicity.



Supplementary Figure S3. Induction of viral escape of HCV genotype 2a culture virus treated with newer PIs. Huh7.5 cells were infected with 2a(JFH1) (2) recombinant virus and treatment with newer PIs was initiated when ~1-20% of culture cells were infected, as determined by HCV-immunostaining. Cell cultures were treated three times per week when cells were split, with 4-, 16-, or 64-fold EC50 of vaniprevir, simeprevir, and grazoprevir, and with 1-, 2-, 4-, 16-, or 64-fold EC50 of faldaprevir, paritaprevir, and deldeprevir The following specific EC50 (nM) values, determined in 48 h high throughput treatment assays (3,5), were used to calculate treatment concentrations: 24 nM for vaniprevir, 73 nM for simeprevir, 16 nM for grazoprevir, 50 nM for faldaprevir, 38 nM for paritaprevir, and 13 nM for deldeprevir (3,6) (unpublished). Non-treated control cultures were followed until viruses spread to ≥80% of culture cells. Treated cultures were followed until the peak in the percentage of HCV-positive culture cells was observed (typically ≥50%), potentially representing viral escape, or until no HCV-positive cells were identified in six consecutive immunostainings, defined as viral suppression. For cultures showing viral spread, the first day following initiation of treatment with ≥80% (non-treated cultures) or ≥50% (treated cultures) HCV-positive cells is reported. For cultures showing viral suppression, the first day

following initiation of treatment with no HCV-positive cells is reported. Data shown in this graph was not necessarily obtained from the same experiment.



65 **Supplementary Figure S4. Boceprevir treatment resulted in decreased viral replication across**
66 **multiple HCV genotypes, which was rescued by key resistance substitutions at NS3P positions**
67 **155 and 156.** RNA transcripts from the indicated 1a(TN)-, 2a(JFH1)-, 2a(J6)-, 3a(S52)-, 3a(452)-,
68 and 6a(HK6a)-recombinants were transfected into S29 cells and 4 h later, cultures were treated with
69 the indicated concentrations of boceprevir. Intracellular (IC) (A) and extracellular (EC) (B)
70 infectivity titers and EC Core levels (C) were determined as described in Materials and Methods.
71 To account for possible differences in transfection efficiency, EC Core concentrations at 48 h were
72 normalized to IC Core concentrations at 4 h. The 4a(ED43) and 5a(SA13) recombinants were not

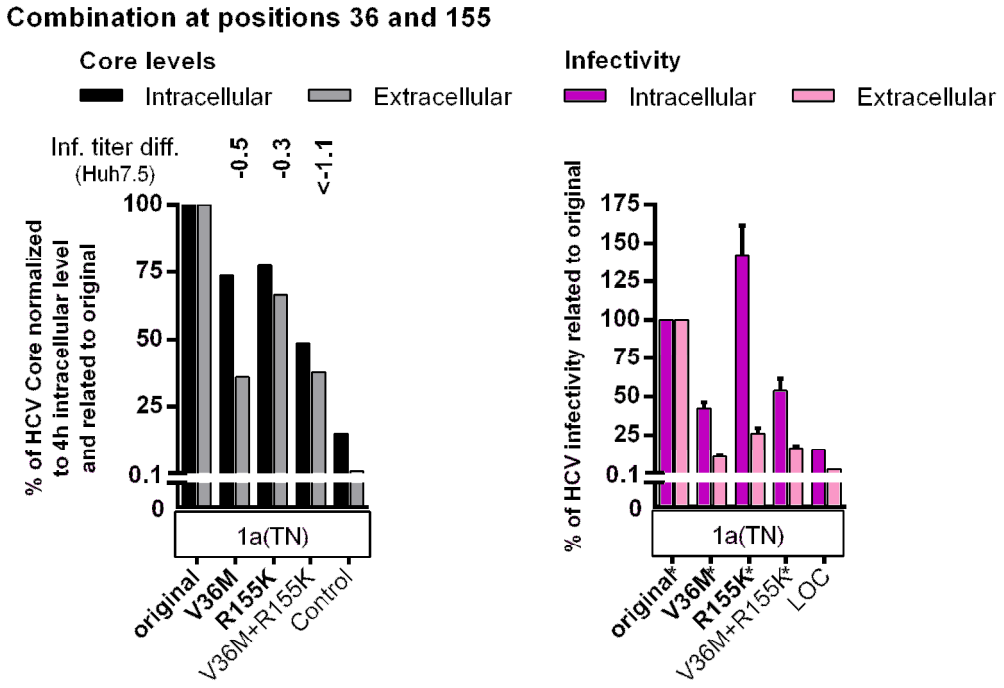
evaluated due to low levels of replication in S29 cells. Transfections of recombinants of the same genotype(isolate) were done in the same experiment.

Control indicates the replication-deficient 2a(JFH1)GND negative control virus, which was included in each transfection experiment. For this control, EC Core concentrations at 48 h ranged from 17.5 to 131.8 fmol/L. The values shown were normalized to 4 h IC Core values as described for NS3P variants.

The breaks in the y-axis indicate the lower cut-off (LOC) of the infectivity titration assay. For automated counting of FFUs, the LOC for IC and EC infectivity titers was 1.5 $\log_{10}$(FFU/well) and 2.3 $\log_{10}$(FFU/ml), respectively. In instances where low replication efficiency in S29 cells precluded automated counting, FFUs were counted manually, and the resulting titers are indicated with an asterisk (*). The LOC for infectivity titers derived from manually counted FFUs was 0.9 $\log_{10}$(FFU/well) for IC titers and 1.6 $\log_{10}$(FFU/ml) for EC titers. Of note, EC infectivity titers might be affected by residual amounts of PI in culture supernatant, especially for less fit recombinants.

The NS3P substitutions in bold were specifically selected for in the indicated virus under PI treatment in the current study (Figure 1).

IC Core levels determined in these experiments are shown in Figure 3.

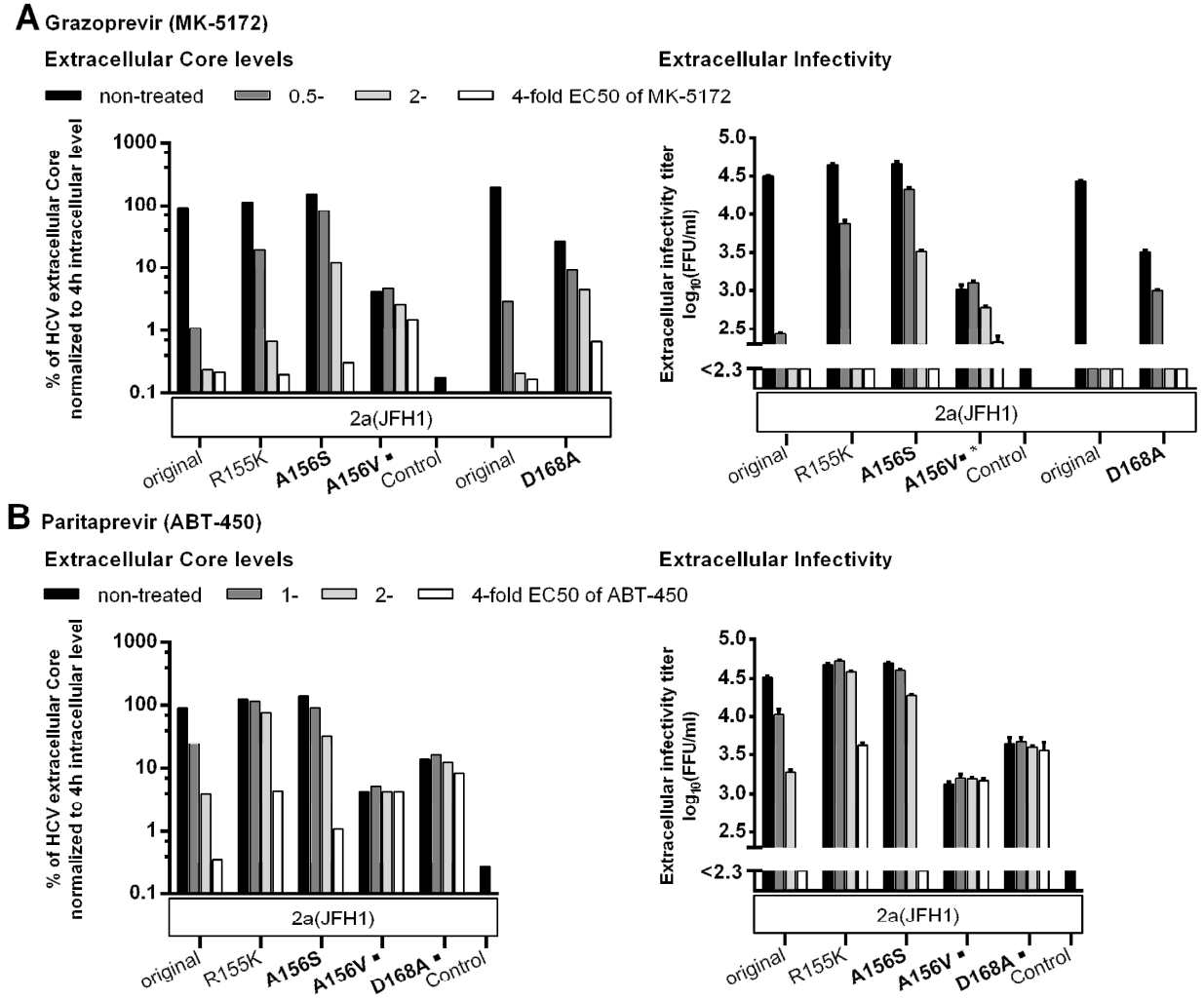


Supplementary Figure S5. Combination of the substitutions V36M and R155K and fitness in the context of the full viral life cycle. The effect of the combination of V36M+R155K substitutions on the viral life cycle was studied by transfection of RNA transcripts from the indicated 1a(TN) recombinants into S29 cells, followed by the determination of intracellular (IC) and extracellular (EC) Core concentrations and infectivity titers as described in Materials and Methods. To account for possible differences in transfection efficiency, IC and EC Core concentrations at 48 h were normalized to IC Core concentrations at 4 h. To determine the effect of the indicated NS3P substitutions on viral fitness, normalized Core values and infectivity titers of variants were related to values of the respective original recombinants (original).

Control indicates the replication-deficient 2a(JFH1)GND negative control virus. For this control, IC Core concentrations at 48 h ranged from 9.4 x 10^3 to 25.0 x 10^3 fmol/L, and EC Core concentrations at 48 h ranged from 17.5 to 131.8 fmol/L. The values shown were normalized to the IC Core concentrations at 4 h and related to the values obtained for the respective original recombinants (original) as was described for the NS3P-variants.

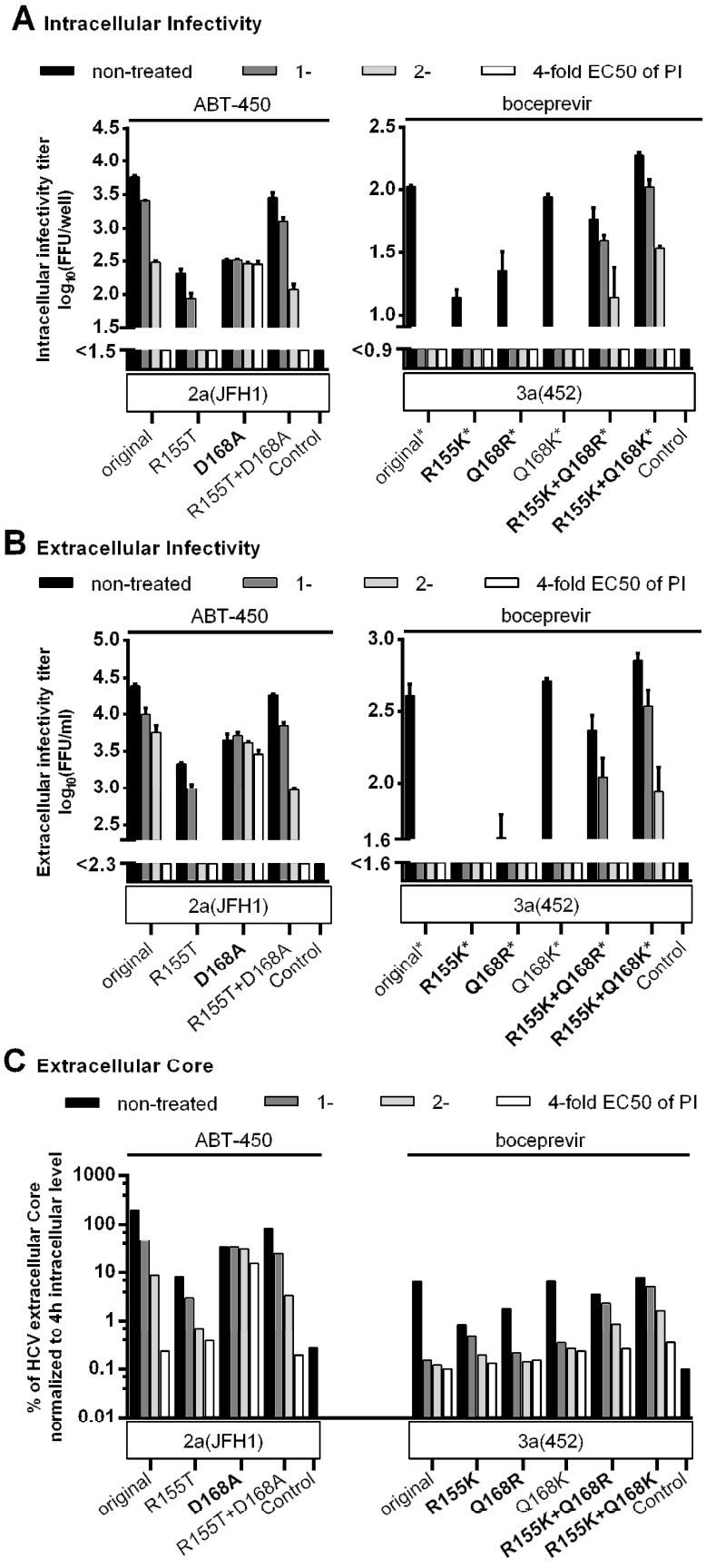
LOC indicates the lower cut-off of the infectivity titration assay. FFUs were counted manually, and the resulting titers are indicated with an asterisk (*). The LOC for infectivity titers derived from manually counted FFUs was 0.9 $\log_{10}$(FFU/well) for IC titers and 1.6 $\log_{10}$(FFU/ml) for EC titers. LOC values were related to the values obtained for the respective original recombinants (original) as described for NS3P variants.

The NS3P substitutions in bold were specifically selected for in the indicated virus under PI treatment in the current study (Figure 1 and Supplementary Table S2). For comparison, titer differences (Inf. titer diff.) observed following transfection of Huh7.5 cells are indicated above each variant. Titer differences were calculated using the titers determined in this study (Table 1) as described in Materials and Methods.



Supplementary Figure S6. Key resistance substitutions at NS3P positions 155, 156 and 168 rescued 2a(JFH1) replication under treatment with newer PIs. RNA transcripts from the indicated 2a(JFH1)-recombinants were transfected into S29 cells and 4 h later, cultures were treated with the indicated concentrations of grazoprevir (MK-5172) (A) or paritaprevir (ABT-450) (B). Extracellular (EC) Core concentrations and infectivity titers were determined as described in Materials and Methods. To account for possible differences in transfection efficiency, EC Core concentrations at 48 h were normalized to IC Core concentrations at 4 h. Transfections of recombinants treated with paritaprevir were done in the same experiment. Transfections of

125 recombinants treated with grazoprevir were done in two different experiments. For each
126 experiment, the original 2a(JFH1) recombinant was included.
127 Control indicates the replication-deficient 2a(JFH1)GND negative control virus. For this control,
128 EC Core concentrations at 48 h ranged from 17.5 to 131.8 fmol/L. The values shown were
129 normalized to 4 h IC Core values as described for the NS3P variants.
130 The breaks in the y-axis indicate the lower cut-off (LOC) of the infectivity titration assay. For
131 automated counting of FFUs, the LOC for EC infectivity titers was 2.3 $\log_{10}$(FFU/ml). For
132 2a(JFH1)A156V treated with grazoprevir, low replication efficiency in S29 cells precluded
133 automated counting. FFUs were counted manually, and the resulting titers are indicated with an
134 asterisk (*). The LOC for infectivity titers derived from manually counted FFUs was 1.6
135 $\log_{10}$(FFU/ml) for EC titers.
136 NS3P substitutions in bold were specifically selected for in the indicated virus under PI treatment in
137 the current study. ■, the substitution was identified in escape variants emerging under treatment
138 with the PI used for treatment (Figure 2).
139 IC Core levels and infectivity titers determined in these experiments are shown in Figure 5.
140


A Intracellular Infectivity
non-treated
1-
2-
4-fold EC50 of PI
ABT-450
boceprevir
Intracellular infectivity titer log10(FFU/well)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
<1.5
2.5
2.0
1.5
1.0
<0.9
2a(JFH1)
3a(452)
original
R155T
D168A
R155T+D168A
Control
original*
R155K*
Q168R*
Q168K*
R155K+Q168R*
R155K+Q168K*
Control
B Extracellular Infectivity
non-treated
1-
2-
4-fold EC50 of PI
ABT-450
boceprevir
Extracellular infectivity titer log10(FFU/ml)
5.0
4.5
4.0
3.5
3.0
2.5
<2.3
3.0
2.5
2.0
1.6
<1.6
2a(JFH1)
3a(452)
original
R155T
D168A
R155T+D168A
Control
original*
R155K*
Q168R*
Q168K*
R155K+Q168R*
R155K+Q168K*
Control
C Extracellular Core
non-treated
1-
2-
4-fold EC50 of PI
ABT-450
boceprevir
% of HCV extracellular Core normalized to 4h intracellular level
1000
100
10
1
0.1
0.01
2a(JFH1)
3a(452)
original
R155T
D168A
R155T+D168A
Control
original
R155K
Q168R
Q168K
R155K+Q168R
R155K+Q168K
Control

Supplementary Figure S7. Combinations of substitutions at positions 155 and 168 increased viral fitness but not PI resistance. RNA transcripts from the indicated 2a(JFH1)- and 3a(452)-recombinants were transfected into S29 cells. Four 4 h later, cultures were treated with the indicated concentrations of paritaprevir (ABT-450) or boceprevir. Intracellular (IC) (A) and extracellular (EC) (B) infectivity titers and EC Core levels (C) were determined as described in Materials and Methods. To account for possible differences in transfections efficiency, EC Core values were normalized to IC Core concentrations at 4 h. Transfections of recombinants of the same genotype(isolate) were done in the same experiment.

Control indicates the replication-deficient 2a(JFH1)GND negative control virus. For this control, EC Core concentrations at 48 h ranged from 17.5 to 131.8 fmol/L. The values shown were normalized to 4 h IC Core values as described for NS3P variants.

The breaks in the y-axis indicate the lower cut-off (LOC) of the infectivity titration assay. For automated counting of FFUs, the LOC for IC and EC infectivity titers was up to 1.5 $\log_{10}$(FFU/well) and 2.3 $\log_{10}$(FFU/ml), respectively. In instances where low replication efficiency in S29 cells precluded automated counting, FFUs were counted manually, and the resulting titers are indicated with an asterisk (*). The LOC for infectivity titers derived from manually counted FFUs was 0.9 $\log_{10}$(FFU/well) for IC titers and 1.6 $\log_{10}$(FFU/ml) for EC titers. Of note, EC infectivity titers (B) might be affected by residual amounts of PI in culture supernatant, especially for less fit recombinants.

The NS3P substitutions in bold were specifically selected in the indicated virus under PI treatment in the current study (Figures 1 and 2, Supplementary Tables S14 and S22).

IC Core levels determined in these experiments are shown in Figure 6.

Supplementary Table S1. Primers and conditions used for the amplification of NS3-NS4A from HCV genotypes 1-6

Primer name	Sequence (5'→ 3')	H77 abs ref[a] (5'→ 3')
2a(JFH1), 2a(J6), 3a(S52), 3a(452), 5a(SA13), and 6a(HK6a) recombinants		
Reverse transcription		
9470R(24)_JFH1	CTATGGAGTGTACCTAGTGTGTGC	9405-9382
First round PCR		
JF3365	CGACTTGGTCGGGAGGTCC	3354-3372
JFH15548	GATCTTGGACTTCAACATCTCGGCTATC	5537-5510
Second round PCR		
JF3382	CCTCCTTGGCCCAGCTGATGG	3371-3391
JFH1R5520	CGCTGCCCCTCTTCGATGAG	5509-5488
1a(TN) full-lenght recombinant		
Reverse transcription		
H9417R	CGTCTCTAGACAGGAAATGGCTTAAGAGGCCGGAGTGTTTACC	9417-9385
First round PCR		
1aF3321	GGTGACATCATCAACGGCTTGC	3321-3342
1aR5541	CGAGGGCCTTCTGCTTGAACTG	5541-5520
Second round PCR		
1aF3391	GAATGGTCTCCAAGGGGTGGAG	3391-3412
1aR5511	GCATCATCCCTTGCTCGATGTACG	5511-5488
4a(ED43) 5'UTR-NS5A recombinant		
Reverse transcription		
9470R(24)_JFH1	CTATGGAGTGTACCTAGTGTGTGC	9405-9382
First round PCR		
ED43F3335	GGATTACCTGTTTCGGCCAGGTTGG	3336-3360
ED43R5564	GCTTGCCAGCGAAATTTAGGAGACC	5565-5541
Second round PCR		
ED43F3360	GCAATGAAATCTTGCTCGGACCAGC	3361-3385
ED43R5528	GCTTGAACTGCTCAGCCAGTTGTAACC	5529-5503

PCR was carried out using BD Advantage 2 Polymerase Mix (Clontech). Cycling conditions for the first round PCR were 99ºC for 35s, followed by 35 cycles of 99ºC for 35s, 67ºC for 30s and 68ºC for 6min, and a final elongation step at 68ºC for 8min. Cycling conditions for second round nested PCR were similar, except the annealing temperature was decreased to 60ºC. For 1a(TN), the annealing temperature used was 67ºC in both first and second round PCR.

173 *a* Primer 5´ to 3´ binding sites are numbered relative to the H77(AF009606) reference sequence.

Supplementary Table S2. Nucleotide changes identified in the NS3P of 1a(TN) at the time of viral escape under telaprevir treatment

Nucleotide position[a]																
1a(TN) plasmid			**3433**	**3525**	**3526**	**3544**	**3552**	**3579**	**3583**	**3604**	**3630**	**3661**	**3796**	**3874**	**3883**	**3885**	**3927**	
H77 rel ref			**14**	**106**	**107**	**125**	**133**	**160**	**164**	**185**	**211**	**242**	**377**	**455**	**464**	**466**	**508**	
H77 abs ref			3433	3525	3526	3544	3552	3579	3583	3604	3630	3661	3796	3874	3883	3885	3927	
1a(TN) nucleotide identity[b]			**C**	**G**	**T**	**C**	**G**	**A**	**T**	**G**	**G**	**A**	**T**	**G**	**G**	**G**	**A**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]																**Subclonal analysis**[f]
NT	**1**	**4**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
NT	**1**	**6**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
0.75x	**1**	**9**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
0.75x	**1**	**11**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
1x	**1**	**9**	•	•	•	•	**G/a**	•	**C**	•	•	**A/g**	•	•	•	•	•	
1x	**1**	**11**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
2x	**1**	**16**	•	•	•	•	•	•	**T/C**	•	•	•	•	•	•	•	•	
2x	**1**	**18**	**C/g**	•	•	•	•	**A/G**	**T/c**	•	**G/a**	•	•	•	•	•	•	
4x	**1**	**16**	•	•	•	•	•	•	**T/C**	•	•	•	**T/c**	**G/c**	•	•	•	
4x	**1**	**18**	•	•	•	•	•	**A/G**	•	•	•	•	•	•	•	•	•	
16x	**1**	**30**	•	**G/A**	**T/c**	•	•	**G**	•	•	•	•	•	•	•	•	•	
16x	**1**	**32**	•	**G/A**	•	•	•	**G**	•	•	•	•	**T/c**	•	**G/A**	•	•	**T54A (1); V36M+T54A (1); V36M+T54A+R155K (2); T54A+R155K (10)**
64x	**1**	**62**	•	•	•	**A**	•	•	•	•	•	•	•	•	•	**A**	**A/g**	
64x	**1**	**65**	•	•	•	**A**	•	•	•	**G/a**	•	•	•	•	•	**A**	•	
Amino acid position[g]																		
H77 rel ref			**5**	**36**	**36**	**42**	**45**	**54**	**55**	**62**	**71**	**81**	**126**	**152**	**155**	**156**	**170**	
H77 abs ref			1031	1062	1062	1068	1071	1080	1081	1088	1097	1107	1152	1178	1181	1182	1196	
1a(TN) amino acid change[h]			**A→G**	**V→M**	**V→A**	**T→N**	**A→T**	**T→A**	**V→A**	**R→K**	**V→I**	**D→G**	**L→P**	**G→A**	**R→K**	**A→T**	**I→V**	

NS3P sequences were obtained from viruses in supernatants from PI treated or non-treated cultures (NT) at the peak of infection of the escape experiment (Supplementary Figure S2).

a Nucleotide positions are numbered according to the given recombinant genome or relative to the H77(AF009606) reference sequence, as specified.

b Nucleotide identity at the respective position of the original recombinant or of viral genomes recovered from infected cell cultures. Coding mutations identified by nucleotide changes that were detected as minor or major variants in at least one time point are shown. The amino acid substitutions estimated to be present in at least 50% of viral genomes are indicated by capital letters, while amino acid substitutions estimated to be present in a minor percentage of viral genomes are indicated by lowercase letters. Dots indicate that the nucleotide at the specified position was conserved.

c Fold EC50 of PI under which the indicated nucleotide changes identified at viral escape were selected. EC50 values of the original viruses were previously determined and are indicated in Supplementary Figure S2.

d Experimental identifier of the escape experiment from which the reported sequence is derived.

e Sequences were from the indicated day following initiation of treatment at which peak infection was observed.

f Subclonal analysis was done as described in the Materials and Methods; typically ~10 clones were analysed. The individual substitutions or combinations of substitutions are shown with the number of identified clones in parentheses. Original, indicates clones for which none of the specified NS3 protease mutations were seen.

g Amino acid positions are numbered relative to the H77(AF009606) reference strain.

h Amino acid changes encoded by the given nucleotide changes are indicated.

Supplementary Table S3. Nucleotide changes identified in the NS3P of 2a(JFH1) at the time of viral escape under telaprevir treatment

Nucleotide position[a]																
2a(JFH1) plasmid			**3449**	**3465**	**3548**	**3573**	**3575**	**3590**	**3630**	**3639**	**3710**	**3798**	**3896**	**3897**	**3938**	**3953**	
H77 rel ref			**19**	**35**	**118**	**143**	**145**	**160**	**200**	**209**	**280**	**368**	**466**	**467**	**508**	**523**	
H77 abs ref			3438	3454	3537	3562	3564	3579	3619	3628	3699	3787	3885	3886	3927	3942	
2a(JFH1) nucleotide identity[b]			**G**	**G**	**T**	**T**	**T**	**A**	**T**	**C**	**T**	**G**	**G**	**C**	**A**	**C**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]															**Subclonal analysis**[f]
NT	**1**	**4**	•	•	•	•	•	•	•	**C/g**	**T/g**	•	•	•	•	•	
NT	**1**	**6**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
NT	**2**	**5**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
NT	**2**	**7**	•	**G/a**	•	•	•	•	•	•	•	•	•	•	•	•	
0.75x	**2**	**12**	**G/a**	•	•	•	•	•	•	•	•	•	•	•	•	•	
0.75x	**2**	**14**	•	•	**T/a**	**T/a**	**T/a**	**A/g**	•	•	•	•	•	•	**A/G**	•	**T54A (1); I170V (5); T54A+I170V (1); original (3)**
1x	**2**	**12**	•	•	**T/a**	**T/a**	**T/a**	**A/g**	•	•	•	**g/A**	•	•	•	**C/t**	
1x	**2**	**14**	•	•	**T/a**	**T/a**	**T/a**	**A/g**	•	•	•	•	•	•	•	•	
2x	**2**	**16**	•	•	•	•	•	**A/g**	•	•	•	•	**g/T**	•	•	•	**T54A (3); A156S (5); original (2)**
2x	**2**	**19**	•	•	•	•	•	•	•	•	•	•	**T**	•	•	•	
4x	**1**	**15**	•	•	•	•	•	•	•	•	•	•	**G/t**	**c/T**	•	•	
4x	**1**	**18**	•	•	•	•	•	•	•	•	•	•	•	**c/T**	•	•	
16x	**1**	**15**	•	•	•	•	•	•	•	•	•	•	•	**T**	•	•	
16x	**1**	**18**	•	•	•	•	•	•	**T/c**	•	•	•	•	**T**	•	•	
Amino acid position[g]																	
H77 rel ref			**7**	**12**	**40**	**48**	**49**	**54**	**67**	**70**	**94**	**123**	**156**		**170**	**175**	
H77 abs ref			1033	1038	1066	1074	1075	1080	1093	1096	1120	1149	1182		1196	1201	
2a(JFH1) amino acid change[h]			**A→T**	**G→D**	**S→T**	**I→N**	**S→T**	**T→A**	**L→S**	**P→R**	**L→V**	**R→Q**	**A→S**[i]	**A→V**[i]	**I→V**	**L→F**	

For details, see footnote of Supplementary Table S2.

i, 2a(JFH1) nucleotide positions 3896 and 3897 are in the the same codon; indicated nucleotide changes encode the indicated amino acid changes at amino acid position 156.

Supplementary Table S4. Nucleotide changes identified in the NS3P of 2a(J6) at the time of viral escape under telaprevir treatment

Nucleotide position[a]									
2a(J6) plasmid			**3590**	**3689**	**3723**	**3762**	**3896**	**3959**	
H77 rel ref			**160**	**259**	**293**	**332**	**466**	**529**	
H77 abs ref			3579	3678	3712	3751	3885	3948	
2a(J6) nucleotide identity[b]			**A**	**A**	**C**	**C**	**G**	**A**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]							**Subclonal analysis**[f]
NT	**1**	**2**	•	•	**C/a**	•	•	•	
NT	**1**	**4**	•	•	**C/A**	•	•	•	
NT	**2**	**4**	•	•	**C/a**	•	•	•	
NT	**2**	**6**	•	**A/c**	•	•	•	•	
0.75x	**2**	**13**	**A/g**	•	**C/A**	•	•	**A/g**	**T98K (4); I177V (1); T54A+T98K (2); original (1)**
0.75x	**2**	**15**	**A/g**	•	**C/A**	•	•	**A/g**	
1x	**1**	**15**	**A/G**	•	**C/A**	•	•	•	
1x	**1**	**20**	**A/G**	•	**C/A**	•	•	•	
2x	**2**	**24**	**G**	•	**c/A**	•	•	**A/g**	
2x	**2**	**26**	**a/G**	•	**A**	•	•	•	
4x	**1**	**74**	**A/t**	•	•	**C/t**	**T**	**G**	
4x	**1**	**76**	**A/t**	•	•	•	**T**	**a/G**	**A156S (2); A156S+I177V (10); T54S+A156S+I177V (1)**
Amino acid position[g]									
H77 rel ref			**54**	**87**	**98**	**111**	**156**	**177**	
H77 abs ref			1080	1113	1124	1137	1182	1203	
2a(J6) amino acid change[h]			**T→A/S**[i]	**S→R**	**T→K**	**A→V**	**A→S**	**I→V**	

For details, see footnote of Supplementary Table S2.

i, A3590G results in T54A; A3590T results in T54S.

Supplementary Table S5. Nucleotide changes identified in the NS3P of 3a(S52) at the time of viral escape under telaprevir treatment

Nucleotide position[a]								
3a(S52) plasmid			**3590**	**3660**	**3666**	**3722**	**3894**	
H77 rel ref			**160**	**230**	**236**	**292**	**464**	
H77 abs ref			3579	3649	3655	3711	3883	
3a(S52) nucleotide identity[b]			**A**	**A**	**A**	**G**	**G**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]						**Subclonal analysis**[f]
NT	**1**	**4**	•	•	•	•	•	
NT	**1**	**6**	•	•	•	•	•	
NT	**2**	**4**	•	•	•	•	•	
NT	**2**	**6**	•	•	•	•	•	
0.75x	**2**	**22**	•	•	•	•	•	
0.75x	**2**	**29**	•	•	**G**	•	•	
1x	**1**	**22**	**A/G**	**A/g**	•	**G/a**	•	**T54A (1); A98T (3); T54A+A98T (3); original (2)**
1x	**1**	**28**	**A/G**	**A/g**	•	**G/a**	•	
2x	**2**	**27**	•	**a/G**	•	•	•	
2x	**2**	**29**	•	**G**	•	•	•	
4x	**1**	**31**	•	**A/g**	•	**G/a**	**g/A**	
4x	**1**	**33**	•	**A/g**	•	**G/a**	**g/A**	**R155K (10): original (1)**
Amino acid position[g]								
H77 rel ref			**54**	**77**	**79**	**98**	**155**	
H77 abs ref			1080	1103	1105	1124	1181	
3a(S52) amino acid change[h]			**T→A**	**N→S**	**D→G**	**A→T**	**R→K**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S6. Nucleotide changes identified in the NS3P of 3a(452) at the time of viral escape under telaprevir treatment

Nucleotide position[a]						
3a(452) plasmid			**3894**	**3896**	**3939**	
H77 rel ref			**464**	**466**	**509**	
H77 abs ref			3883	3885	3928	
3a(452) nucleotide identity[b]			**G**	**G**	**T**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]				**Subclonal analysis**[f]
NT	**1**	**2**	•	•	•	
NT	**1**	**4**	•	•	•	
NT	**2**	**4**	•	•	•	
NT	**2**	**6**	•	•	•	
0.75x	**2**	**22**	•	**G/T**	**T/C**	**A156S (1); I170T (3)**
0.75x	**2**	**25**	•	**G/T**	**T/c**	
1x	**1**	**31**	**G/A**	**G/T**	•	
1x	**1**	**33**	**G/A**	**G/T**	•	**R155K (3); A156S (8)**
2x	**2**	**42**	**A**	•	•	
2x	**2**	**48**	**A**	•	•	
Amino acid position[g]						
H77 rel ref			**155**	**156**	**170**	
H77 abs ref			1181	1182	1196	
3a(452) amino acid change[h]			**R→K**	**A→S**	**I→T**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S7. Nucleotide changes identified in the NS3P of 4a(ED43) at the time of viral escape under telaprevir treatment

Nucleotide position[a]											
4a(ED43) plasmid			**3459**	**3648**	**3683**	**3702**	**3734**	**3756**	**3812**	**3881**	**3884**	**3936**
H77 rel ref			**41**	**230**	**265**	**284**	**316**	**338**	**395**	**463**	**466**	**518**
H77 abs ref			3460	3649	3684	3703	3735	3757	3814	3882	3885	3937
4a(ED43) nucleotide identity[b]			**T**	**A**	**C**	**C**	**C**	**T**	**A**	**C**	**G**	**A**
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]										
NT	**1**	**4**	•	•	•	•	•	•	•	•	•	•
NT	**1**	**7**	•	•	•	•	•	•	•	•	•	•
0.75x	**1**	**11**	**T/g**	•	•	**C/a**	**C/a**	•	•	•	•	•
0.75x	**1**	**16**	•	•	•	•	•	•	•	•	•	•
1x	**1**	**14**	•	•	•	•	•	**T/c**	•	•	•	•
1x	**1**	**16**	•	•	•	•	•	•	**A/g**	•	•	•
2x	**1**	**53**	•	**a/G**	•	•	•	•	**A/g**	•	**G/t**	•
2x	**1**	**58**	•	**A/G**	•	•	•	•	•	•	**G/T**	•
4x	**1**	**73**	•	•	**C/g**	•	•	•	•	**C/t**	**T**	**A/c**
4x	**1**	**78**	•	•	•	•	•	•	•	•	**T**	•
Amino acid position[f]												
H77 rel ref			**14**	**77**	**89**	**95**	**106**	**113**	**132**	**155**	**156**	**173**
H77 abs ref			1040	1103	1115	1121	1132	1139	1158	1181	1182	1199
4a(ED43) amino acid change[g]			**F→C**	**N→S**	**P→A**	**A→D**	**L→I**	**V→A**	**I→V**	**R→C**	**A→S**	**E→A**

NS3P sequences were obtained from viruses in supernatants from PI treated or non-treated cultures (NT) at the peak of infection of the escape experiment (Supplementary Figure S2).

a Nucleotide positions are numbered according to the given recombinant genome or relative to the H77(AF009606) reference sequence, as specified.

b Nucleotide identity at the respective position of the original recombinant or of viral genomes recovered from infected cell cultures. Coding mutations identified by nucleotide changes that were detected as minor or major variants in at least one time point are shown. The amino acid substitutions estimated to be present in at least 50% of viral genomes are indicated by capital letters, while amino acid substitutions estimated to be present in a minor percentage of viral genomes are indicated by lowercase letters. Dots indicate that the nucleotide at the specified position was conserved.

c Fold EC50 of PI under which the indicated nucleotide changes identified at viral escape were selected. EC50 values of the original viruses were previously determined and are indicated in Supplementary Figure S2.

d Experimental identifier of the escape experiment, from which the reported sequence is derived.

e Sequences were from the indicated day following initiation of treatment at which peak infection was observed.

f Amino acid positions are numbered relative to the H77(AF009606) reference strain.

g Amino acid changes encoded by the given nucleotide changes are indicated.

Supplementary Table S8. Nucleotide changes identified in the NS3P of 5a(SA13) at the time of viral escape under telaprevir treatment

Nucleotide position[a]							
5a(SA13) plasmid			**3593**	**3780**	**3894**	**3896**	
H77 rel ref			**163**	**350**	**464**	**466**	
H77 abs ref			3582	3769	3883	3885	
5a(SA13) nucleotide identity[b]			**G**	**G**	**G**	**G**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]					**Subclonal analysis**[f]
NT	**1**	**2**	•	•	•	•	
NT	**1**	**4**	•	•	•	•	
NT	**2**	**4**	•	•	•	•	
NT	**2**	**6**	•	•	•	•	
0.75x	**2**	**22**	•	•	**A**	•	
0.75x	**2**	**25**	**G/t**	•	**A**	•	
1x	**1**	**24**	•	•	**G/A**	**G/T**	**R155K (3); A156S (4)**
1x	**1**	**26**	•	•	**g/A**	•	
2x	**2**	**36**	•	•	**g/A**	**G/t**	
2x	**2**	**39**	•	•	**G/a**	**g/T**	
4x	**1**	**38**	•	**G/t**	•	**T**	
4x	**1**	**43**	•	•	•	**T**	
Amino acid position[g]							
H77 rel ref			**55**	**117**	**155**	**156**	
H77 abs ref			1081	1143	1181	1182	
5a(SA13) amino acid change[h]			**V→F**	**R→L**	**R→K**	**A→S**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S9. Nucleotide changes identified in the NS3P of 6a(HK6a) at the time of viral escape under telaprevir treatment

Nucleotide position[a]										
6a(HK6a) plasmid			**3537**	**3552**	**3558**	**3590**	**3782**	**3837**	**3896**	
H77 rel ref			**107**	**122**	**128**	**160**	**352**	**407**	**466**	
H77 abs ref			3526	3541	3547	3579	3771	3826	3885	
6a(HK6a) nucleotide identity[b]			**T**	**A**	**T**	**A**	**C**	**A**	**G**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]								**Subclonal analysis**[f]
NT	**1**	**4**	•	•	•	•	**C/g**	**A/g**	•	
NT	**1**	**7**	•	**A/t**	•	•	•	•	•	
NT	**2**	**2**	•	•	•	•	•	•	•	
NT	**2**	**4**	•	•	•	•	**C/g**	•	•	
NT	**3**	**5**	•	•	•	•	•	•	•	
NT	**3**	**7**	•	•	•	•	•	•	•	
0.75x	**3**	**7**	•	•	•	•	•	•	•	
0.75x	**3**	**19**	•	•	•	•	•	•	•	
1x	**2**	**15**	•	•	•	**G**	**C/g**	•	•	
1x	**2**	**18**	•	•	**T/g**	**A/G**	**C/g**	•	•	**F43C (1); T54A (9)**
2x	**3**	**19**	**T/C**	•	•	**A/G**	**C/g**	•	•	**V36A (5); T54A (3); original (1)**
2x	**3**	**21**	•	•	•	**A/g**	•	•	•	
4x	**1**	**39**	•	•	•	•	•	•	**g/T**	
4x	**1**	**42**	•	•	•	**A/g**	**C/g**	•	**g/T**	**A156S (9)**
Amino acid position[g]										
H77 rel ref			**36**	**41**	**43**	**54**	**118**	**136**	**156**	
H77 abs ref			1062	1067	1069	1080	1144	1162	1182	
6a(HK6a) amino acid change[h]			**V→A**	**Q→L**	**F→C**	**T→A**	**R→G**	**K→R**	**A→S**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S10. Nucleotide changes identified in the NS3P of 1a(TN) at the time of viral escape under boceprevir treatment

Nucleotide position[a]										
1a(TN) plasmid			**3433**	**3471**	**3472**	**3579**	**3583**	**3630**	**3883**	
H77 rel ref			**14**	**52**	**53**	**160**	**164**	**211**	**464**	
H77 abs ref			3433	3471	3472	3579	3583	3630	3883	
1a(TN) nucleotide identity[b]			**C**	**A**	**T**	**A**	**T**	**G**	**G**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]								**Subclonal analysis**[f]
NT	**1**	**4**	•	•	•	•	•	•	•	
NT	**1**	**6**	•	•	•	•	•	•	•	
0.75x	**1**	**9**	•	•	•	•	**T/C**	•	•	
0.75x	**1**	**11**	**C/g**	•	•	•	**T/C**	•	•	
1x	**1**	**13**	•	•	•	**A/g**	**T/C**	•	•	
1x	**1**	**16**	•	**A/g**	•	**A/g**	**T/C**	**G/A**	•	**I18V (2); V55A (1); T54A+V71I (2); V55A+V71I (4)**
2x	**1**	**16**	•	•	•	**a/G**	**T/c**	•	•	
2x	**1**	**18**	•	•	•	**A/G**	**T/c**	•	•	
4x	**1**	**20**	•	•	•	**G**	•	•	•	
4x	**1**	**23**	•	•	•	**G**	•	•	•	
16x	**1**	**57**	•	•	**C**	•	•	•	**C**	
16x	**1**	**61**	•	•	**C**	•	•	•	**C**	
Amino acid position[g]										
H77 rel ref			**5**	**18**		**54**	**55**	**71**	**155**	
H77 abs ref			1031	1044		1080	1081	1097	1181	
1a(TN) amino acid change[h]			**A→G**	**I→V**[i]	**I→T**[i]	**T→A**	**V→A**	**V→I**	**R→T**	

For details, see footnote of Supplementary Table S2.

i, 1a(TN) nucleotide positions 3471 and 3472 are in the same codon; indicated nucleotide changes encode the indicated amino acid changes at amino acid position 18.

Supplementary Table S11. Nucleotide changes identified in the NS3P of 2a(JFH1) at the time of viral escape under boceprevir treatment

Nucleotide position[a]									
2a(JFH1) plasmid			**3465**	**3590**	**3824**	**3861**	**3897**	**3953**	
H77 rel ref			**35**	**160**	**394**	**431**	**467**	**523**	
H77 abs ref			3454	3579	3813	3850	3886	3942	
2a(JFH1) nucleotide identity[b]			**G**	**A**	**A**	**T**	**C**	**C**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]							**Subclonal analysis**[f]
NT	**1**	**4**	•	•	•	•	•	•	
NT	**1**	**6**	•	•	•	•	•	•	
NT	**2**	**5**	•	•	•	•	•	•	
NT	**2**	**7**	**G/a**	•	•	•	•	•	
0.75x	**2**	**12**	•	**A/g**	•	•	•	•	
0.75x	**2**	**14**	•	**A/g**	•	•	•	•	
1x	**2**	**13**	•	**A/G**	•	•	•	**C/t**	
1x	**2**	**15**	•	**A/g**	•	•	•	**C/T**	
2x	**2**	**16**	•	**A/g**	•	•	**C/T**	•	
2x	**2**	**19**	•	**A/g**	•	**T/g**	**C/T**	•	**T54A (3); A156V (4)**
4x	**1**	**15**	•	•	•	•	**T**	•	
4x	**1**	**17**	•	•	•	•	**T**	•	
16x	**1**	**38**	•	•	•	•	**T**	•	
16x	**1**	**40**	•	•	**A/g**	•	**T**	•	
Amino acid position[g]									
H77 rel ref			**12**	**54**	**132**	**144**	**156**	**175**	
H77 abs ref			1038	1080	1158	1170	1182	1201	
2a(JFH1) amino acid change[h]			**G→D**	**T→A**	**I→V**	**L→R**	**A→V**	**L→F**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S12. Nucleotide changes identified in the NS3P of 2a(J6) at the time of viral escape under boceprevir treatment

Nucleotide position[a]										
2a(J6) plasmid			**3474**	**3483**	**3590**	**3645**	**3689**	**3723**	**3959**	
H77 rel ref			**44**	**53**	**160**	**215**	**259**	**293**	**529**	
H77 abs ref			3463	3472	3579	3634	3678	3712	3948	
2a(J6) nucleotide identity[b]			**G**	**T**	**A**	**C**	**A**	**C**	**A**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]								**Subclonal analysis**[f]
NT	**1**	**2**	•	•	•	•	•	**C/a**	•	
NT	**1**	**4**	•	•	•	•	•	**C/A**	•	
NT	**2**	**4**	•	•	•	•	•	**C/a**	•	
NT	**2**	**6**	•	•	•	•	**A/c**	•	•	
0.75x	**2**	**13**	•	•	**A/g**	•	•	**C/a**	•	
0.75x	**2**	**15**	•	•	**A/g**	•	•	**C/A**	•	
1x	**1**	**28**	•	•	**A/G**	**C/t**	•	**c/A**	•	
1x	**1**	**31**	•	•	**a/G**	**C/t**	•	**C/A**	•	**T54A (9); T72M (3)**
2x	**2**	**24**	**G/a**	**T/c**	**G**	•	•	**c/A**	**A/g**	**T54A+T98K (5); T98K (1)**
2x	**2**	**26**	•	**T/c**	**a/G**	•	•	**c/A**	**A/g**	
Amino acid position[g]										
H77 rel ref			**15**	**18**	**54**	**72**	**87**	**98**	**177**	
H77 abs ref			1041	1044	1080	1098	1113	1124	1203	
2a(J6) amino acid change[h]			**G→D**	**V→A**	**T→A**	**T→M**	**S→R**	**T→K**	**I→V**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S13. Nucleotide changes identified in the NS3P of 3a(S52) at the time of viral escape under boceprevir treatment

Nucleotide position[a]							
3a(S52) plasmid			**3590**	**3594**	**3660**	**3894**	
H77 rel ref			**160**	**164**	**230**	**464**	
H77 abs ref			3579	3583	3649	3883	
3a(S52) nucleotide identity[b]			**A**	**T**	**A**	**G**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]					**Subclonal analysis**[f]
NT	**1**	**4**	•	•	•	•	
NT	**1**	**6**	•	•	•	•	
NT	**2**	**4**	•	•	•	•	
NT	**2**	**6**	•	•	•	•	
0.75x	**2**	**25**	**A/G**	**T/c**	•	**G/a**	**T54A (6); R155K (2); original (2)**
0.75x	**2**	**27**	**A/g**	•	**A/g**	•	
1x	**1**	**22**	**A/G**	•	•	**G/a**	**T54A (3); R155K (2)**
1x	**1**	**29**	**A/G**	•	•	•	
2x	**2**	**40**	•	•	•	**A**	
2x	**2**	**42**	•	•	•	**A**	
4x	**1**	**35**	•	•	•	**A**	
4x	**1**	**40**	•	•	•	**A**	
Amino acid position[g]							
H77 rel ref			**54**	**55**	**77**	**155**	
H77 abs ref			1080	1081	1103	1181	
3a(S52) amino acid change[h]			**T→A**	**V→A**	**N→S**	**R→K**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S14. Nucleotide changes identified in the NS3P of 3a(452) at the time of viral escape under boceprevir treatment

Nucleotide position[a]							
3a(452) plasmid			**3894**	**3926**	**3933**	**3939**	
H77 rel ref			**464**	**496**	**503**	**509**	
H77 abs ref			3883	3915	3922	3928	
3a(452) nucleotide identity[b]			**G**	**G**	**A**	**T**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]					**Subclonal analysis**[f]
NT	**1**	**4**	•	•	•	•	
NT	**1**	**6**	•	•	•	•	
0.75x	**1**	**22**	•	•	•	•	
0.75x	**1**	**25**	•	•	•	**T/C**	
1x	**1**	**27**	**G/a**	**G/a**	•	**T/c**	**R155K (1); A166T (5); I170T (7)**
1x	**1**	**29**	•	•	•	**C**	
2x	**1**	**57**	**g/A**	**G/a**	•	•	**R155K (2); R155K+Q168K (1); A166T+Q168R (1)**
2x	**1**	**60**	**g/A**	**g/A**	**a/G**	•	**R155K (2); R155K+Q168R (2); A166T+Q168R (4)**
Amino acid position[g]							
H77 rel ref			**155**	**166**	**168**	**170**	
H77 abs ref			1181	1192	1194	1196	
3a(452) amino acid change[h]			**R→K**	**A→T**	**Q→R**	**I→T**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S15. Nucleotide changes identified in the NS3P of 4a(ED43) at the time of viral escape under boceprevir treatment

Nucleotide position[a]						
4a(ED43) plasmid			**3645**	**3648**	**3743**	**3884**
H77 rel ref			**227**	**230**	**325**	**466**
H77 abs ref			3646	3649	3744	3885
4a(ED43) nucleotide identity[b]			**C**	**A**	**A**	**G**
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]				
NT	**1**	**4**	•	•	•	•
NT	**1**	**7**	•	•	•	•
0.75x	**1**	**11**	•	•	•	•
0.75x	**1**	**18**	•	•	**A/g**	•
1x	**1**	**53**	**C/t**	•	•	**G/T**
1x	**1**	**60**	**C/t**	•	•	**G/T**
2x	**1**	**60**	•	**G**	•	•
2x	**1**	**62**	•	**G**	•	•
Amino acid position[f]						
H77 rel ref			**76**	**77**	**109**	**156**
H77 abs ref			1102	1103	1135	1182
4a(ED43) amino acid change[g]			**T→I**	**N→S**	**R→G**	**A→S**

For details, see footnote of Supplementary Table S7.

Supplementary Table S16. Nucleotide changes identified in the NS3P of 5a(SA13) at the time of viral escape under boceprevir treatment

Nucleotide position[a]								
5a(SA13) plasmid			**3482**	**3519**	**3591**	**3894**	**3896**	
H77 rel ref			**52**	**89**	**161**	**464**	**466**	
H77 abs ref			3471	3508	3580	3883	3885	
5a(SA13) nucleotide identity[b]			**A**	**A**	**C**	**G**	**G**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]						**Subclonal analysis**[f]
NT	**1**	**2**	•	•	•	•	•	
NT	**1**	**4**	•	•	•	•	•	
NT	**2**	**4**	•	•	•	•	•	
NT	**2**	**6**	•	•	•	•	•	
0.75x	**2**	**25**	•	•	**c/G**	**A**	•	
0.75x	**2**	**29**	•	•	**C/g**	**A**	•	
1x	**1**	**26**	**A/g**	•	•	**G/A**	**G/t**	**R155K (4); A156S (7); I18V+R155K (3)**
1x	**1**	**29**	**A/g**	•	•	**G/A**	**G/t**	
2x	**2**	**45**	•	**A/t**	•	•	**T**	
2x	**2**	**49**	•	•	•	•	**T**	
Amino acid position[g]								
H77 rel ref			**18**	**30**	**54**	**155**	**156**	
H77 abs ref			1044	1056	1080	1181	1182	
5a(SA13) amino acid change[h]			**I→V**	**E→V**	**T→S**	**R→K**	**A→S**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S17. Nucleotide changes identified in the NS3P of 6a(HK6a) at the time of viral escape under boceprevir treatment

Nucleotide position[a]													
6a(HK6a) plasmid			**3552**	**3590**	**3633**	**3782**	**3821**	**3837**	**3855**	**3896**	**3902**	**3942**	
H77 rel ref			**122**	**160**	**203**	**352**	**391**	**407**	**425**	**466**	**472**	**512**	
H77 abs ref			3541	3579	3622	3771	3810	3826	3844	3885	3891	3931	
6a(HK6a) nucleotide identity[b]			**A**	**A**	**A**	**C**	**C**	**A**	**C**	**G**	**G**	**C**	
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]											**Subclonal analysis**[f]
NT	**1**	**2**	•	•	•	•	•	•	•	•	•	•	
NT	**1**	**4**	•	•	•	**C/g**	•	•	•	•	•	•	
NT	**2**	**5**	•	•	•	•	•	•	•	•	•	•	
NT	**2**	**7**	•	•	•	•	•	•	•	•	•	•	
0.75x	**2**	**14**	•	**a/G**	•	•	•	•	•	•	•	•	
0.75x	**2**	**16**	•	**A/G**	•	•	•	•	•	•	•	•	
1x	**1**	**15**	•	**a/G**	•	•	•	•	•	•	•	•	
1x	**1**	**18**	•	**a/G**	•	•	•	•	•	•	•	**C/t**	**T54A (6); original (2)**
2x	**2**	**30**	•	**G**	**A/g**	•	**C/t**	•	•	•	•	•	
2x	**2**	**33**	•	**G**	•	•	•	•	**C/g**	•	•	•	
4x	**1**	**53**	•	**G**	•	•	•	•	•	**G/T**	**G/A**	•	
4x	**1**	**55**	•	**G**	•	•	•	•	•	**G/T**	**G/A**	•	**T54A+A156S (4); T54A+V158I (3)**
Amino acid position[g]													
H77 rel ref			**41**	**54**	**68**	**118**	**131**	**136**	**142**	**156**	**158**	**171**	
H77 abs ref			1067	1080	1094	1144	1157	1162	1168	1182	1184	1197	
6a(HK6a) amino acid change[h]			**Q→L**	**T→A**	**K→R**	**R→G**	**P→S**	**K→R**	**P→R**	**A→S**	**V→I**	**P→L**	

For details, see footnote of Supplementary Table S2.

Supplementary Table S18. Nucleotide changes identified in the NS3P of 2a(JFH1) at the time of viral escape under vaniprevir (MK-7009) treatment

Nucleotide position[a]					
2a(JFH1) plasmid			**3897**	**3900**	**3933**
H77 rel ref			**467**	**470**	**503**
H77 abs ref			3886	3889	3922
2a(JFH1) nucleotide identity[b]			**C**	**C**	**A**
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]			
NT	**1**	**5**	•	•	•
NT	**1**	**7**	•	•	•
4x	**1**	**13**	•	•	**a/c/g/t**
4x	**1**	**15**	•	•	**a/c/g/t**
16x	**1**	**15**	**C/t**	•	**a/C/g/t**
16x	**1**	**17**	**C/t**	**C/t**	**a/C/g/t**
64x	**1**	**20**	**c/T**	•	**A/t**
64x	**1**	**22**	**C/T**	•	**A/t**
Amino acid position[f]					
H77 rel ref			**156**	**157**	**168**
H77 abs ref			1182	1183	1194
2a(JFH1) amino acid change[g]			**A→V**	**A→V**	**D→A/G/V**[h]

For details, see footnote of Supplementary Table S7.

c Fold EC50 of PI under which the indicated nucleotide changes identified at viral escape were selected. EC50 values of the original viruses were previously determined and are indicated in Supplementary Figure S3.

h, A3933C results in D168A; A3933G results in D168G; A3933T results in D168V.

Supplementary Table S19. Nucleotide changes identified in the NS3P of 2a(JFH1) at the time of viral escape under faldaprevir (BI 201335) treatment

Nucleotide position[a]							
2a(JFH1) plasmid			3657	3896	3897	3933	3939
H77 rel ref			**227**	**466**	**467**	**503**	**509**
H77 abs ref			3646	3885	3886	3922	3928
2a(JFH1) nucleotide identity[b]			**C**	**G**	**C**	**A**	**T**
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]					
NT	**1**	**5**	•	•	•	•	•
NT	**1**	**7**	•	•	•	•	•
1x	**1**	**12**	•	•	•	•	•
1x	**1**	**16**	•	•	•	•	•
2x	**1**	**16**	•	•	•	**A/C/t**	•
2x	**1**	**19**	•	•	•	**A/C/t**	•
4x	**1**	**15**	**C/t**	•	•	**A/t**	**T/c**
4x	**1**	**19**	•	•	•	**a/T**	•
16x	**1**	**14**	•	**G/a**	•	•	•
16x	**1**	**19**	•	**G/A**	•	•	•
64x	**1**	**33**	•	•	**T**	•	•
64x	**1**	**35**	•	•	**T**	•	•
Amino acid position[f]							
H77 rel ref			**76**	**156**	**156**	**168**	**170**
H77 abs ref			1102	1182	1182	1194	1196
2a(JFH1) amino acid change[g]			**S→L**	**A→T**	**A→V**	**D→A/V**[h]	**I→T**

For details, see footnote of Supplementary Table S7.

c Fold EC50 of PI under which the indicated nucleotide changes identified at viral escape were selected. EC50 values of the original viruses were previously determined and are indicated in Supplementary Figure S3.

h, A3933C results in D168A; A3933T results in D168V.

Supplementary Table S20. Nucleotide changes identified in the NS3P of 2a(JFH1) at the time of viral escape under simeprevir (TMC435350) treatment

Nucleotide position[a]							
2a(JFH1) plasmid			**3596**	**3627**	**3666**	**3932**	**3933**
H77 rel ref			**166**	**197**	**236**	**502**	**503**
H77 abs ref			3585	3616	3655	3921	3922
2a(JFH1) nucleotide identity[b]			**T**	**G**	**A**	**G**	**A**
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]					
NT	**1**	**4**	•	•	•	•	•
NT	**1**	**6**	•	**G/a**	•	•	•
4x	**1**	**11**	**T/c**	•	**A/g**	•	•
16x	**1**	**8**	•	•	•	**G/t**	**A/c/T**
16x	**1**	**11**	•	•	•	•	**A/c/T**
64x	**1**	**15**	•	•	•	•	**T**
64x	**1**	**18**	•	•	•	•	**T**
Amino acid position[f]							
H77 rel ref			**56**	**66**	**79**	**168**	**168**
H77 abs ref			1082	1092	1105	1194	1194
2a(JFH1) amino acid change[g]			**Y→H**	**G→D**	**E→G**	**D→Y**[h]	**D→A/V**[i]

For details, see footnote of Supplementary Table S7.

c Fold EC50 of PI under which the indicated nucleotide changes identified at viral escape were selected. EC50 values of the original viruses were previously determined and are indicated in Supplementary Figure S3.

h, 2a(JFH1) nucleotide positions 3932 and 3933 are in the same codon; indicated nucleotide changes encode the indicated amino acid changes at amino acid position 168.

i, A3933C results in D168A; A3933T results in D168V.

Supplementary Table S21. Nucleotide changes identified in the NS3P of 2a(JFH1) at the time of viral escape under deldeprevir (ACH-2684) treatment

Nucleotide position[a]						
2a(JFH1) plasmid			**3596**	**3666**	**3897**	**3933**
H77 rel ref			**166**	**236**	**467**	**503**
H77 abs ref			3585	3655	3886	3922
2a(JFH1) nucleotide identity[b]			**T**	**A**	**C**	**A**
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]				
NT	**1**	**5**	•	•	•	•
NT	**1**	**7**	•	•	•	•
1x	**1**	**7**	•	•	•	•
1x	**1**	**10**	•	•	•	•
2x	**1**	**10**	**T/c**	**A/g**	•	•
2x	**1**	**12**	**T/c**	**A/g**	•	•
4x	**1**	**12**	**T/c**	•	•	**A/C**
4x	**1**	**14**	**T/c**	•	•	**A/C**
16x	**1**	**12**	•	•	•	**A/c/T**
16x	**1**	**17**	•	•	•	**A/c/T**
64x	**1**	**14**	•	•	**C/T**	**A/t**
64x	**1**	**19**	•	•	**C/T**	**A/t**
Amino acid position[f]						
H77 rel ref			**56**	**79**	**156**	**168**
H77 abs ref			1082	1105	1182	1194
2a(JFH1) amino acid change[g]			**Y→H**	**E→G**	**A→V**	**D→A/V**[h]

For details, see footnote of Supplementary Table S7.

c Fold EC50 of PI under which the indicated nucleotide changes identified at viral escape were selected. EC50 values of the original viruses were previously determined and are indicated in Supplementary Figure S3.

h, A3933C results in D168A; A3933T results in D168V.

Supplementary Table S22. Nucleotide changes identified in the NS3P of 2a(JFH1) at the time of viral escape under paritaprevir (ABT-450) treatment

Nucleotide position[a]								
2a(JFH1) plasmid			**3437**	**3557**	**3596**	**3630**	**3795**	**3897**	**3933**
H77 rel ref			**7**	**127**	**166**	**200**	**365**	**467**	**503**
H77 abs ref			3426	3546	3585	3619	3784	3886	3922
2a(JFH1) nucleotide identity[b]			**A**	**T**	**T**	**T**	**A**	**C**	**A**
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]							
NT	**1**	**5**	•	•	•	•	•	•	•
NT	**1**	**7**	•	•	•	•	•	•	•
1x	**1**	**9**	•	•	•	•	•	•	•
1x	**1**	**14**	•	•	•	•	•	•	•
2x	**1**	**14**	•	•	**T/c**	•	**a/C**	**C/T**	•
4x	**1**	**14**	•	•	**T/C**	•	•	•	**A/C**
4x	**1**	**16**	•	•	**T/C**	•	•	•	**A/C**
16x	**1**	**19**	•	**G**	•	**C**	•	•	•
16x	**1**	**21**	•	**G**	•	**C**	•	•	•
16x	**1**	**23**	**A/g**	**G**	•	**C**	•	•	•
Amino acid position[f]									
H77 rel ref			**3**	**43**	**56**	**67**	**122**	**156**	**168**
H77 abs ref			1029	1069	1082	1093	1148	1182	1194
2a(JFH1) amino acid change[g]			**I→V**	**F→V**	**Y→H**	**L→S**	**K→T**	**A→V**	**D→A**

For details, see footnote of Supplementary Table S7.

c Fold EC50 of PI under which the indicated nucleotide changes identified at viral escape were selected. EC50 values of the original viruses were previously determined and are indicated in Supplementary Figure S3.

Supplementary Table S23. Nucleotide changes identified in the NS3P of 2a(JFH1) at the time of viral escape under grazoprevir (MK-5172) treatment

Nucleotide position[a]							
2a(JFH1) plasmid			**3630**	**3645**	**3705**	**3897**	**3934**
H77 rel ref			**200**	**215**	**275**	**467**	**504**
H77 abs ref			3619	3634	3694	3886	3923
2a(JFH1) nucleotide identity[b]			**T**	**C**	**A**	**C**	**T**
Fold EC_{50}[c]	**Exp**[d]	**Day**[e]					
NT	**1**	**4**	•	•	•	•	•
NT	**1**	**6**	•	•	•	•	•
4x	**1**	**13**	•	•	•	**T**	•
4x	**1**	**15**	•	•	•	**T**	•
16x	**1**	**20**	•	•	**A/G**	**T**	•
16x	**1**	**22**	•	•	**A/G**	**T**	•
64x	**1**	**43**	**T/c**	**C/T**	•	**T**	•
64x	**1**	**46**	**T/c**	**C/t**	•	**T**	**T/g**
Amino acid position[f]							
H77 rel ref			**67**	**72**	**92**	**156**	**168**
H77 abs ref			1093	1098	1118	1182	1194
2a(JFH1) amino acid change[g]			**L→S**	**T→M**	**K→R**	**A→V**	**D→E**

For details, see footnote of Supplementary Table S7.

c Fold EC50 of PI under which the indicated nucleotide changes identified at viral escape were selected. EC50 values of the original viruses were previously determined and are indicated in Supplementary Figure S3.

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