

1 **Table S1.** Listing of HCV replicons used to characterize pan-genotype potency of grazoprevir  
 2 and elbasvir

Replicon Constructs	Accession Number					References for Replicon Background
	NS3/4A full length <sup>a</sup>	NS3 <sup>pro</sup> (1-181) <sup>b</sup>	NS4A <sup>c</sup>	NS5A <sup>d</sup>	Replicon Background <sup>e</sup>	
GT1a(H77) FL <sup>f</sup>	NC004102			NC004102	GT1a(H77)	(17)
GT1b (Con1) FL	AJ238799			AJ238799	GT1b (Con1)	(18)
GT2a (JFH-1) FL	AB047639			AB047639	GT2a (JFH-1)	(20)
GT2b(MD2b6-1) NS3 <sup>g</sup>		AY232740	AY232740		GT2a (JFH-1)	
GT2b(JPUT971017) NS5A <sup>g</sup> M31				AB030907	GT2a (JFH-1)	
GT2b(JPUT971017) NS5A M31L				AB030907	GT2a (JFH-1)	
GT3a(GLA) NS3		GU945445	GU945457.1		GT2a (JFH-1)	
GT3a(S52) NS3		GU814263	GU814263		GT2a (JFH-1)	
GT3a(S52) FL	GU814263			GU814263	GT3a(S52)	(21)
GT3a(NZL1) NS5A				NC009824	GT1b (Con1)	
GT4a(ED43) FL	GU814265			GU814265	GT4a(ED43)	(21)
GT5a(SA13) NS3		AF064490	AF064490		GT2a (JFH-1)	
GT5a(SA13) NS5A				AF064490	GT2a (JFH-1)	
GT6a(HK6a) NS3		JN180455.1	JN180455.1		GT1b (N)	(19)
GT6a(GZ52557) NS3		DQ278892	DQ278892		GT2a (JFH-1)	
GT6a(GZ52557) NS5A				DQ278892	GT2a (JFH-1)	

<sup>a</sup> NS3/4A full length: complete NS3 (aa 1-631) and NS4A genes from indicated sequence

<sup>b</sup> NS3pro: NS3 protease catalytic domain (aa 1-181)

<sup>c</sup> NS4A: complete NS4A gene from indicated sequence

<sup>d</sup> NS5A: complete NS5A gene from indicated sequence

<sup>e</sup> Replicon background: sequence of parental subgenomic replicon cDNA

<sup>f</sup> FL: full-length replicon of indicated genotype

<sup>g</sup> NS3 or NS5A: gene from indicated genotype introduced into chimeric genome

4 **Table S2.** Primer and probes sets for real-time RT-PCR of HCV RNA, by genotype  
5

	Primer Sequence (5' to 3')	Position on full-length virus sequence	Accession Number
GT1a (primer/probe set from HCV IRES)			
1a F	TGCGGAACCGGTGAGTACA	149-167	NC004102
1a R	GCGGGTTTATCCAAGAAAGGA	191-211	
1a probe	6FAM-CGGAATTGCCAGGACGACCGG-TAMRA	169-189	
GT1b, GT6a primer/probe set, 5B.2			
Con1 F	ATGGACAGGCGCCCTGA	7613-7629	AJ238799
Con1 R	TTGATGGGCAGCTTGGTTTC	7650-7669	
Con1 Probe	6FAM-CACGCCATGCGCTGCGG-TAMRA	7631-7647	
GT2a, GT2b, GT5a primer/probe set IRES			
JFH F	CGCAAGACTGCTAGCCGAG	237-255	AB047639
JFH R	GCCCTATCAGGCAGTACCACA	279-299	
JFH Probe	6FAM-AGCGTTGGGTTGCGAAAGGCC-TAMRA	257-277	
GT3a primer/probe set IRES			
S52 F	TGCGGAACCGGTGAGTACA	147-165	GU814263
S52 R	GCGGGTTGCTCCAAGAAAGGA	189-209	
S52 Probe	6FAM-CGGAATCGCTGGGGTGACCGG-TAMRA	167-187	
GT4a primer/probe set 5B			
ED43 F	TGAGAGCGACGGCGTAGAG	8563-8581	GU814265
ED43 R	ATCTCGTCATAGCCTCCGTGAA	8606-8627	
ED43 Probe	6FAM-AGGACAACCGAGCCCTCCGAGC-TAMRA	8583-8604	
Program: 48° C for 30 minutes, 95° C for 10 minutes, and 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.			

6

7 **Table S3.** Summary of clonal variants found from untreated (DMSO) control GT1a(H77)  
 8 replicons

9 **A. NS3 protease domain**

AA

NS3	C	D	V	A	Q	Q	G	S	R	A	L	Y	R	A	D	I	N
DMSO	16	25	33	39	41	89	100	101	109	111	127	134	155	156	168	170	174
AA1	C	D	V	A	R	Q	G	S	R	T	L	Y	R	A	D	I	S
AA10	C	D	V	A	R	Q	G	S	R	T	L	Y	R	A	D	I	S
AA3	C	D	V	A	R	Q	G	S	R	A	L	Y	R	A	D	I	S
AA9	C	D	V	A	R	Q	G	S	R	A	L	Y	R	A	D	I	S
AA6	C	D	G	A	R	Q	G	S	R	A	L	Y	R	A	D	I	S
41-1791	C	D	V	A	R	Q	S	S	R	A	L	Y	R	A	D	I	S
41-1788	C	D	V	A	R	Q	G	S	R	A	L	Y	R	A	D	I	S
41-1546	C	D	V	A	R	Q	G	S	R	A	P	Y	R	A	D	I	S
41-6	C	D	V	A	R	Q	G	S	R	A	P	Y	R	A	D	I	S
41-32	C	D	V	A	R	Q	G	S	R	A	L	Y	R	A	D	I	S
41-34	C	D	V	A	R	Q	G	P	G	A	L	Y	R	A	D	I	S
41-1549	R	D	V	A	Q	Q	G	S	R	A	L	Y	R	A	D	I	S
41-1551	C	G	V	A	Q	Q	G	S	R	A	L	Y	R	A	D	I	S
41-19	C	D	V	A	Q	Q	G	S	R	A	L	Y	R	A	D	I	S
41-1548	C	D	V	A	Q	Q	G	S	R	A	L	Y	R	A	D	I	S
41-1789	C	D	V	A	Q	Q	G	S	R	A	L	Y	R	A	D	I	S
41-37	C	D	V	A	Q	Q	G	S	R	A	L	Y	R	A	D	I	S
AA8	C	D	V	T	Q	Q	G	S	R	A	L	H	R	A	D	I	N
41-11	C	D	V	A	Q	Q	G	S	R	V	L	Y	R	A	D	I	N
41-1787	C	D	V	A	Q	R	G	S	R	A	L	Y	R	A	D	I	N

10

11

12 **B. NS5A Domain I**

NS5A	<i>I</i>	<i>E</i>	<i>K</i>	<i>M</i>	<i>Q</i>	<i>L</i>	<i>I</i>	<i>T</i>	<i>H</i>	<i>S</i>	<i>Y</i>
DMSO	12	14	24	28	30	31	52	55	58	85	93
AA1	I	E	K	M	Q	L	I	T	H	S	Y
AA10	I	E	K	M	Q	L	I	T	H	S	Y
AA3	I	E	K	M	Q	L	I	T	H	S	Y
AA9	I	E	K	M	Q	L	I	T	H	S	Y
AA6	I	E	K	M	Q	L	I	T	R	G	Y
41-1791	I	E	K	M	Q	L	I	T	H	S	Y
41-1788	I	E	K	M	Q	L	I	T	H	S	Y
41-1546	I	E	K	M	Q	L	I	T	H	S	Y
41-6	I	E	K	M	Q	L	I	T	H	S	Y
41-32	I	E	K	M	Q	L	V	T	H	S	Y
41-34	I	E	K	M	Q	L	I	T	H	S	Y
41-1549	I	E	Q	M	Q	L	I	T	H	S	Y
41-1551	T	E	K	M	Q	L	I	T	H	S	Y
41-19	I	E	K	M	Q	L	I	T	H	S	Y
41-1548	I	E	K	M	Q	L	I	T	H	S	Y
41-1789	I	E	K	M	Q	L	I	T	H	S	Y
41-37	I	E	K	M	Q	L	I	T	H	S	Y
AA8	I	E	K	M	Q	L	I	A	H	S	Y
41-11	I	E	K	M	Q	L	I	A	H	S	Y
41-1787	I	E	K	M	Q	L	I	T	H	S	Y

13

14 Red letters highlight variations from the sequence template used for alignments (listed at the top of each column). If  
 15 the sequence was not different from the template, it was not included (exception: key RAVs, highlighted in yellow).  
 16 The gene and treatment type are denoted in the upper left corner of each table. Each row starts with the name for a  
 17 sequenced cDNA clone spanning the NS3 to NS5B polyprotein gene.

18 **Table S4.** Variants selected by elbasvir at different EC<sub>90</sub> multiples in GT1a(H77) replicon cells

19

Treatment (EC <sub>90</sub> Multiples)	EBR-selected NS5A variants
1X [5 clones]	Q30R+R81M (60%) K24E+Q30R (40%)
3X [15 clones]	M28T (20%) Q30E (6.7%) Q30R (13.3%) Y93C (33.3%) K24T+Q30R (6.7%) K24T+M28T+Q30R (6.7%) M28T+Q30H (6.7%) Q30R+Y93H (6.7%)
10X [18 clones]	Q30R (5.6%) L31V (22.2%) Y93H (5.6%) K24E+Q30R (5.6%) K24E+L31V (5.6%) K24Q+Y93H (5.6%) K24R+Y93C (5.6%) K24T+Q30R (33.3%) K24T+Y93H (5.6%) K24Q+M28I+L31M (5.6%)
30X [11 clones]	Q30E (9.1%) L31V (9.1%) Y93H (18.2%) K24A+Q30R (9.1%) K24T+Q30R (36.4%) M28T+Q30R (18.2%)
100X [4 clones]	Y93N (100%)
1000X [5 clones]	K24I+M28K (40%) Q30R+L31V (60%)

20 The percentage (in parentheses) indicates the frequency of that variant among the clones  
 21 sequenced for that dose level.

22

23

24

25 Tables S5 and S6 show results of clonal sequencing, in a format that organizes linkage of NS3<sup>pro</sup>  
 26 and NS5A  
 27

28 **Table S5.** Amino Acid Variants found in Combination Therapy Groups with 1x EC<sub>90</sub>  
 29 Grazoprevir

Treatment Multiple	GZR-selected NS3 variants	EBR-selected NS5A variants	Frequency (%)
1X GZR – 0X EBR [10 clones]	wt	wt	10
	Q41R	wt	30
	R155K	wt	10
	Q41R	K24T	30
	Q41R+R155K	wt	10
	Q41R+D168E	wt	10
1X GZR – 1X EBR [14 clones]	Q41R	Q30G	7.1
	R109K	Q30R	14.3
	R109K	K24E+Q30R	7.1
	Q41R+R109K	Q30R	50
	Q41R+R109K	K24E+Q30R	14.3
	Q41R+R109K	L31V	7.1
1X GZR – 3X EBR [9 clones]	Q41R	L31M	44.4
	Q41R	M28K+L31M	11.1
	Q41R	K24T+Q30R	22.2
	Q41R	K24R+L31M	11.1
	Q41R_R155K	L31M+Y93C	11.1
1X GZR – 10X EBR [19 clones]	Q41R	M28T	10.5
	Q41R	Q30E	5.3
	Q41R	Q30D	5.3
	Q41R	K24T+Q30R	10.5
	Q41R	M28T+Y93C	5.3
	D168A+N174E	wt	26.3
	D168A+N174E	M28K	5.3
	Q41R+R109K+N174E	K24E+Q30R	26.3
	Q41R+R109K+N174E	K24T+Q30R	5.3
1X GZR – 100X EBR [3 clones]	R109K	K24G+Q30R	33.3
	R109K	K24R+Q30E	66.7

30 The percentage (in parentheses) indicates the frequency of that variant among the clones  
 31 sequenced for that dose level.

32

33 **Table S6.** Amino Acid Variants found in Combination Therapy Groups with Higher EC<sub>90</sub>  
 34 Multiples of Grazoprevir

Treatment Multiple	GZR-selected NS3 variants	EBR-selected NS5A variants	Frequency (%)
3X GZR – 3X EBR [13 clones]	Q41R+R155K	Q30D	7.7
	Q41R+R155K	K24T+Q30R	23.1
	Q41R+D168E	K24T+Q30R	7.7
	Q41R+D168E	Q30D	38.5
	Q41R+D168E	M28V	15.4
	Q41R+D168E	K24T+M28T+Q30R	7.7
10X GZR – 0X EBR [1 clone]	D168A	wt	100
10X GZR – 1X EBR [14 clones]	D168E	Q30H	7.1
	D168V	Q30H	78.6
	D168S	K24R+Q30H	7.1
	D168V	K24R+Q30H	7.1

35 The percentage (in parentheses) indicates the frequency of that variant among the clones  
 36 sequenced for that dose level.