Supplemental material:

Barrier-independent, fitness-associated differences in sofosbuvir-efficacy against hepatitis C virus

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Table S1. Mutations, and corresponding amino acid of the NS5B-coding region in the consensus sequence of HCV p45 and HCV p100 after five and ten passages in the absence and presence of sofosbuvir (SOF) 800 nM and 1200 nM[a]

HCV population [number of passages][a]	No drug		SOF 800 nM		SOF 1200 nM	
	Mutation[b]	Amino acid substitution[b]	Mutation[b]	Amino acid substitution[b]	Mutation[b]	Amino acid substitution[b]
HCV p45	T7842T/C	V59V/A	-		-	
	C8842T	Syn				
	T8962A	Syn				
HCV p45 [p5]	T7842T/C	V59V/A	T7842C/T	V59A/V	T7842C	V59A
	C8842T	Syn	T8092C	Syn	A8531T	M289L
	T8962A	Syn	A8521G	Syn	C8842T	Syn
			A8531T	M289L	T8962A	Syn
			C8842T	Syn		
			T9380C	Syn		
HCV p45 [p10]	T7842C/T	V59A/V	C7714C/T*	Syn*	T7842C	V59A
	A8427A/G*	H254H/R*	T7842C	V59A	A8201A/G*	T179T/A*
	C8575C/T*	Syn*	T8092C	Syn	A8531T	M289L
	C8842T	Syn	A8521G	Syn	C8842T	Syn
	T8962A	Syn	A8531T	M289L	T8962A	Syn
			C8842T	Syn		
			T9380C	Syn		
HCV p100	T7842T/C*	V59V/A*	-		-	
	A8201G/A	T179A/T				
	A8263G	Syn				
	T8310C	M215T				
	C8842T	Syn				
	C8854A/C	Syn				
	T8962A	Syn				
	T9014G/T	S450A/S				
	C9385G/C	Syn				
HCV p100 [p5]	T7727T/C*	Syn*	T7842T/C*	V59V/A*	C7948C/T	Syn
	T7842T/C*	V59V/A*	A8201G/A	T179A/T	A8201G	T179A
	A8263G	Syn	A8263G	Syn	A8263G	Syn
	T8310C	M215T	T8310C	M215T	T8310C	M215T
	C8842T	Syn	C8842T	Syn	A8530A/T	M289M/L
	T8962A	Syn	C8854A/C	Syn	C8842T	Syn
	T9014G/T	S450A/S	T8962A	Syn	C8854A	Syn
	C9364C/T*	Syn*	T9014G/T	S450A/S	T8962A	Syn
	C9385C/G*	Syn*	C9385G/C	Syn	T9014G	S450A
					C9385G/C	Syn
					C9364C/T*	Syn*
HCV p100 [p10]	C7678C/T*	Syn*	T7842C	V59A	NO VIRUS ISOLATED	
	T7842T/C*	V59V/A*	A8531T	M289L		
	T7942T/C*	Syn*	C8842T	Syn		
	G8209T/G	K181N/K	T8962A	Syn		
	A8263A/G	Syn				
	T8310C/T	M215T/M				
	T8722T/A*	D352D/E*				
	A8758A/G	Syn				

	C8842T	Syn			
	C8854C/A*	Syn*			
	T8884T/C	Syn			
	T8962A	Syn			
	T9014T/G	S450S/A			
	C9364C/T*	Syn*			
	C9385C/G	Syn			

[a] The sequence of the NS5B-coding region was determined for HCV p45 and HCV p100 after 5 and 10 passages in the absence and presence of SOF 800 nM and 1200 nM. The populations are those described in Figures 2 and 3 of the main text.

[b] Mutations and deduced amino acid substitutions are relative to the sequence of the JFH-1 (accession number AB047639). Amino acid residues (single-letter code) are numbered individually for each protein from the N-to the C-terminus. Boldface type indicates a resistant mutation previously described. Two residues separated by a bar indicate a mixture of two nucleotides in the population, according to the sequence band pattern. Asterisks indicate a proportion of the mutant lower than 25%. Procedures for nucleotide sequencing are described in Materials and Methods.

Table S2. Mutations, and corresponding amino acid of the NS5B-coding region in the consensus sequence of HCV p0, HCV p45 and HCV p100 biological clones after one, three or five passages in the absence and presence of sofosbuvir (SOF) 800 nM[a]

HCV population [number of passages][a]	**No drug**		**SOF 800 nM**	
	Mutation[b]	**Amino acid substitution[b]**	**Mutation[b]**	**Amino acid substitution[b]**
HCV p0 CB2	A7696G	Syn	-	
	A8201G	T179A		
HCV p0 CB2 [p3]	A7696G	Syn	A7696G	Syn
	C7714C/T*	Syn*	A8201G	T179A
	A8201G	T179A	G8586G/A*	G307G/D*
HCV p45 CB2	T8200C	Syn	-	
	A8629G	Syn		
	C8842T	Syn		
	T8962A	Syn		
	G9416A	G584S		
HCV p45 CB2 [p3]	T8200C	Syn	T8200C	Syn
	A8629G	Syn	G8499G/A*	R278R/H*
	C8842T	Syn	A8629G	Syn
	T8962A	Syn	C8842T	Syn
	G9416A	G584S	T8962A	Syn
			G9416A	G584S
HCV p45 CB3	C8842T	Syn	-	
	T8962A	Syn		
HCV p45 CB3 [p3]	C8842T	Syn	C8842T	Syn
	T8962A	Syn	T8962A	Syn
HCV p100 CB 4	T7727C	Syn	-	
	C7735T	Syn		
	T7967C	Y101H		
	A8263G	Syn		
	T8310C	M215T		
	C8842T	Syn		
	T8962A	Syn		
	T9014G	S450A		
HCV p100 CB 4 [p3]	T7727C	Syn	T7727C	Syn
	C7735T	Syn	C7735T	Syn
	T7742T/A*	L26L/M*	A7870A/G	Syn
	T7967C	Y101H	T7967C	Y101H
	A8263G	Syn	A8263G	Syn
	T8310C	M215T	T8310C	M215T
	C8842T	Syn	A8531A/T	M289M/L
	T8962A	Syn	C8842T	Syn
	T9014G	S450A	T8962A	Syn
			T9014G	S450A
HCV p100 CB 5	T7727C	Syn	-	
	G7898A	V78I		
	T7954C	Syn		

	A8263G	Syn		
	T8310C	M215T		
	T8314G	Syn		
	A8626G	Syn		
	C8842T	Syn		
	T8962A	Syn		
	T9014G	S450A		
	A9235T	Syn		
HCV p100 CB 5 [p3]	T7727C	Syn	T7727C	Syn
	G7898A	V78I	G7898A	V78I
	T7954C	Syn	T7954C	Syn
	A8263G	Syn	A8263G	Syn
	T8310C	M215T	T8310C	M215T
	T8314G	Syn	T8314G	Syn
	A8626G	Syn	A8626G	Syn
	C8842T	Syn	C8842T	Syn
	T8962A	Syn	T8962A	Syn
	T9014G	S450A	T9014G	S450A
	A9235T	Syn	A9235T	Syn
HCV p100 CB 9	A8263G	Syn	-	
	T8310C	M215T		
	C8842T	Syn		
	T8962A	Syn		
	T9014G	S450A		
	A9217G	Syn		
	C9364T	Syn		
HCV p100 CB 9 [p3]	A8263G	Syn	T7855C	Syn
	T8310C	M215T	A8263G	Syn
	C8842T	Syn	T8310C	M215T
	T8962A	Syn	A8531T	M289L
	T9014G	S450A	C8842T	Syn
	A9217G	Syn	T8962A	Syn
	C9364T	Syn	T9014G	S450A
			A9217G	Syn
			C9364T	Syn

[a] The sequence of the NS5B-coding region was determined for HCV p0, HCV p45 and HCV p100 biological clones after 3 passages in the absence and presence of SOF 800 nM[a]. The populations are those described in Figures 4 and 5 of the main text.

[b] Mutations and deduced amino acid substitutions are relative to the sequence of the JFH-1 (accession number AB047639). Amino acid residues (single-letter code) are numbered individually for each protein from the N-to the C-terminus. Boldface type

indicates a resistant mutation previously described. Two residues separated by a bar indicate a mixture of two nucleotides in the population, according to the sequence band pattern. Asterisks indicate a proportion of the mutant lower than 25%. Procedures for nucleotide sequencing are described in Materials and Methods.