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Supplemental information

Sites of vulnerability in HCV E1E2 identified by comprehensive functional screening

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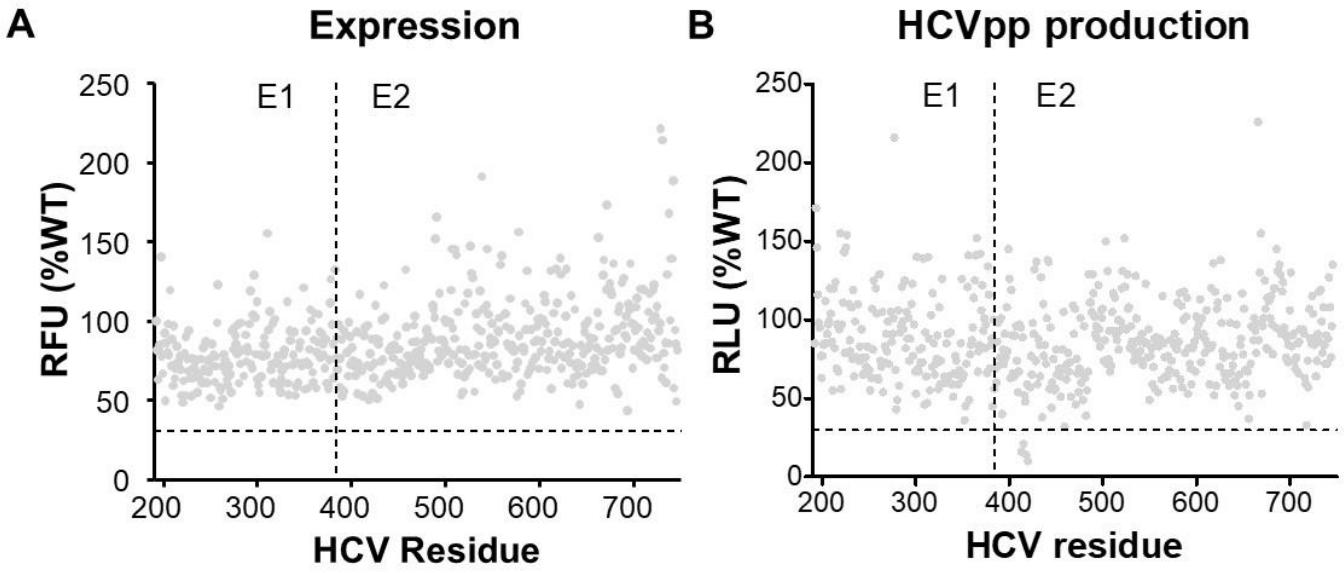


Figure S1. Mutations do not affect full-length E1E2 expression or incorporation into HCVpp. Related to Figures 2 and 3. Each clone of the HCV E1E2 library was transfected into HEK-293T cells. The C-terminus of E2 was constructed with a V5 tag. **(A)** Full-length E1E2 protein expression was analyzed by intracellular staining with an anti-V5 antibody. **(B)** E1E2 protein was incorporated onto pseudoviruses (HCVpp) by co-expression of E1E2 with HIV Gag-pol core and a luciferase reporter genome. Supernatants from each mutant clone were analyzed by ELISA. A mouse anti-CD81 antibody was used to capture pseudovirus particles, and linear human MAb HCV1 was used to detect E1E2 incorporation, values shown are the mean of two replicates. A chemiluminescence assay using an HRP-conjugated rabbit anti-human secondary antibody served as the readout for E1E2 incorporation. Clones with mutations in the known epitope of MAb HCV1 were omitted from the analysis.

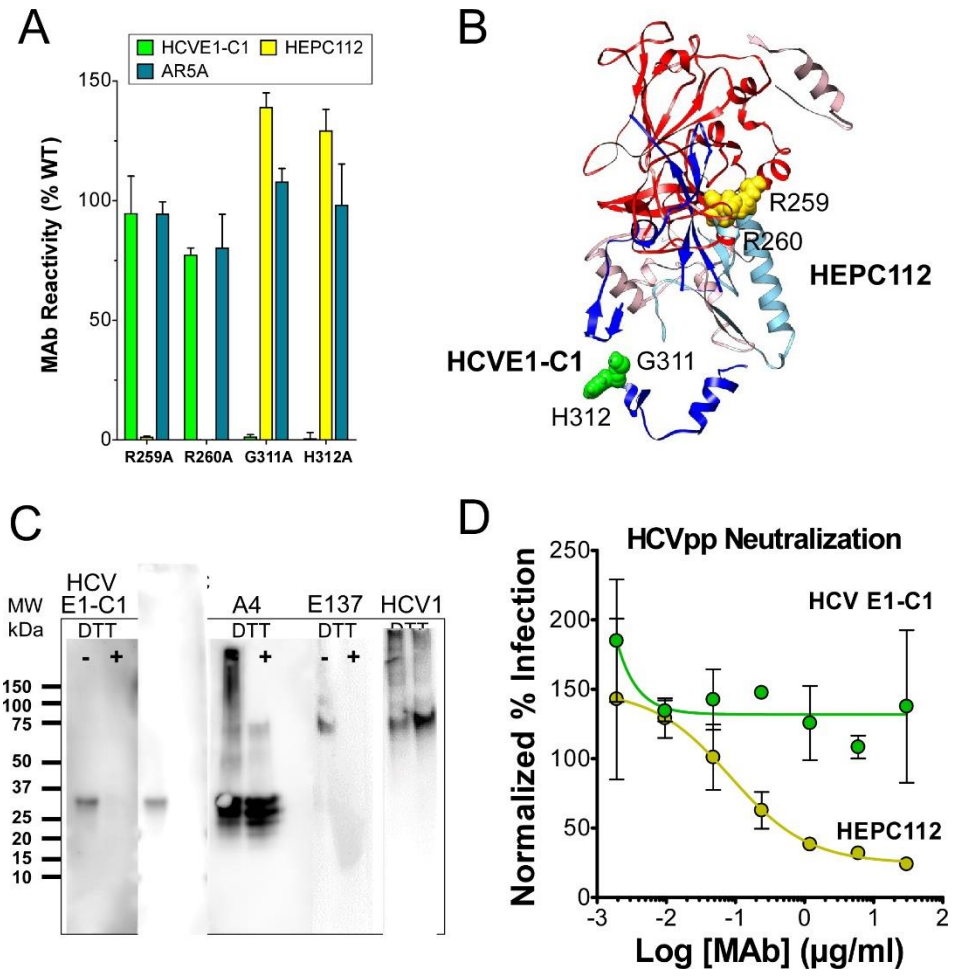
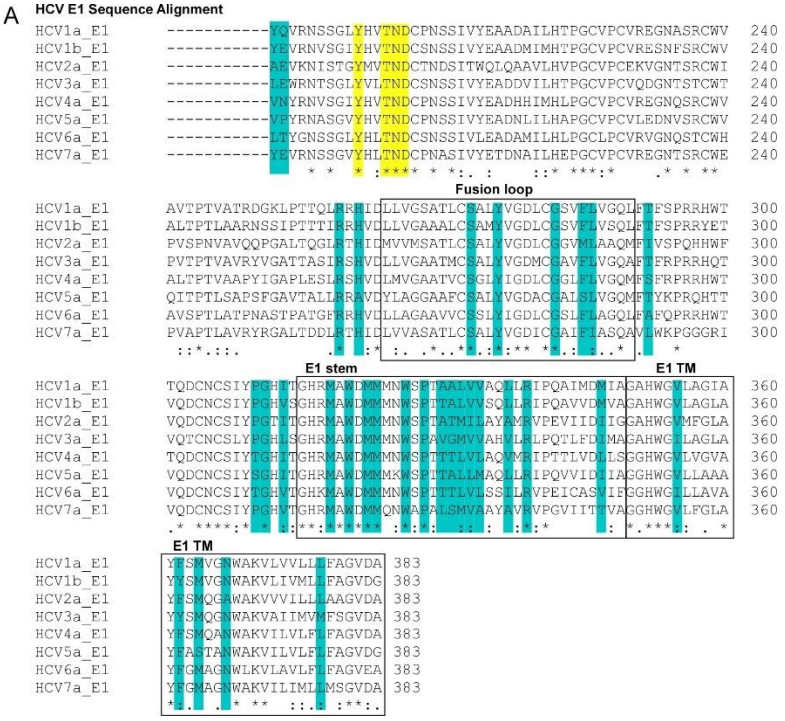


Figure S2. Characterization of conformational anti-E1 MAbs. Related to Figure 1. (**A**) Binding to critical mutations is shown for E1 MAbs HCVE1-C1 and HEPC112 (Colbert et al., 2019). MAb AR5A is shown as a control, as it binds only when E1 and E2 are in complex and is sensitive to mutations that perturb E1 structure (Giang et al., 2012). Binding values are given as a percentage of MAb binding to wild-type HCV E1E2 and are plotted with error bars showing half the range (highest minus lowest binding value) of at least two measurements. (**B**) The critical residues for HCVE1-C1 (G311 and H312) are shown as green spheres located in the transmembrane proximal region of E1 (PDB 2KNU). The critical epitope residues of HEPC112 (R259 and R260) are shown as yellow spheres, as reported previously (Colbert et al., 2019). **(C)** Conformational dependence of HCVE1-C1, HEPC112, and other MAbs was determined by Western analysis. Each well was loaded with 5 µg of protein from the lysate of HCV E1E2-expressing cells, followed by boiling in SDS-PAGE buffer without (-) or with DTT (+). MAbs HCVE1-C1, HEPC112 and A4 target E1, while MAbs E137 and HCV1 target E2. MAbs A4 and HCV1 recognize non-conformational epitopes, so were included as controls. **(D)** Neutralization by anti-E1 MAbs HCVE1-C1 and HEPC112 were assayed by incubation with wild-type HCVpp prior to infection of HEK-293T cells expressing Claudin 1. Infectivity values were normalized to the value for infectivity obtained without antibody. Values represent the mean of 2 replicates, with error bar showing the range.

A


HCV E1 Sequence Alignment
Fusion loop
E1 stem
E1 TM
E1 TM

B


HCV E2 Sequence Alignment
HVR1
cNTR
VR2
VR3
Stem region
E2 TM

Figure S3. Sequence conservation of HCV Env polyprotein across HCV genotypes. Related to Figure 3. (A) Sequence alignment for HCV E1 glycoprotein. **(B)** Sequence alignment for HCV E2 glycoprotein. Critical residues for E1E2 assembly are highlighted in yellow, those for infectivity in blue. ClustalW alignment was performed using parental sequence HCV1a isolate H77 together with reference sequences for each genotype recommended by the International Committee on Taxonomy of Viruses. Regions of structure- and sequence-related importance are denoted. Subtype/genotype, isolate/locus, accession # (reference): 1b, HPCJCG, D90208 (Kato et al., 1990); 2a, HPCPOLP, D00944 (Han and Houghton, 1992; Hotta et al., 1994; Okamoto et al., 1991); 3a, HPCEGS, D17763 (Sakamoto et al., 1994); 4a, ED43, Y11604 (Chamberlain et al., 1997a); 5a, EUH1480, Y13184 (Chamberlain et al., 1997b); 6a, EUHK2, Y12083 (Adams et al., 1997); 7a, QC69, EF108306 (Murphy et al., 2015).

Table S1. Antibodies used in this study. Related to Figures 2, 3

MAb (Ref.)	Epitope			Neutralization Ability						
	Type	Region	Critical Residues	Test	Tested Genotypes	Cross-reactivity	Fraction of 1a Strains Neutralized[a]	Potency	Mechanism	Class
Broadly Neutralizing MAbs										
AP33 (Owsianka et al., 2005)	NC	E2 cNTR	L413, N415, G418, W420	HCVpp	1a, 1b, 2a, 2b, 3-6	All tested	N/D	High	CD81 interaction	bNAb
AR2A (Giang et al., 2012; Law et al., 2008)	C	E2 AR2	K628	HCVpp, HCVcc	1a, 1b, 2a, 2b, 3-6	1a, 2a, 2b, 4-5	N/D	Variable	Post-attachment	bNAb
AR3A (Giang et al., 2012; Law et al., 2008)	C	E2 AR3	T425, N428, G436, W437, L438, F442, Y485, W529, G530, D535, W616	HCVpp, HCVcc	1a, 1b, 2a, 2b, 3-6	All tested	N/D	Variable	CD81 binding, pre-/post-attachment	bNAb
AR3B (Law et al., 2008)	C	E2 AR3	T425, L427, N428, W437, G440, F442, D520, W529, G530, D535, W616	HCVpp, HCVcc	1a, 1b, 2a, 2b, 4-5	All tested	N/D	Variable	CD81 binding	bNAb
AR3C (Law et al., 2008)	C	E2 AR3	T425, N428, W437, L438, F442, Y443, W529, G530, D535, W616	HCVpp, HCVcc	1a, 1b, 2a, 2b, 4-5	All tested	N/D	Variable	CD81 binding	bNAb
AR3D (Law et al., 2008)	C	E2 AR3	T425, L427, N428, G436, W437, G440, F442, D520, W529, G530, D535, W616	HCVpp, HCVcc	1a, 1b, 2a, 2b, 4-5	All tested	N/D	Variable	CD81 binding	bNAb
AR4A (Torrents de la Peña et al., 2021)	C	E2 stem	I696	HCVpp, HCVcc	1a, 1b, 2a, 2b, 3-6	1a, 2a, 4a, 5a, 6a	N/D	Variable	Pre-/post-attachment	bNAb
AR5A (Giang et al., 2012)	C	E2 stem	K628, R630, L665	HCVpp, HCVcc	1a, 1b, 2a, 2b, 3-6	1a, 1b, 2a, 4-6	N/D	Variable	Pre-/post-attachment	bNAb
e20 (Castelli et al., 2017; Mancini et al., 2009)	C	E2 AR3	T425, L427, N428, W437, F442, W529, G530, W616	HCVpp, HCVcc	1a, 1b, 2a, 2b, 4-5	1a, 1b, 2a, 2b, 4	N/D	Variable	CD81 binding	bNAb
e137 (Castelli et al., 2017; Perotti et al., 2008)	C	E2 AR3	T425, N428, N430, S432, W437, L438, G530	HCVpp, HCVcc	1a, 1b, 2a, 2b, 3-6	1a, 1b, 2b, 4	N/D	Variable	CD81 binding	bNAb
H77.39 (Sabo et al., 2011)	NC	E2 cNTR	N415, G418, W420	FFU assay	1-6	1-5	N/D	High	CD81 and SR-B1 binding early post-attachment	bNAb
HCV1 (Broering et al., 2009; Kong et al., 2012)	NC	E2 cNTR	L413, G418, W420	HCVpp	1a, 1b, 2b, 3a, 4a	All tested	N/D	High	CD81 binding	bNAb
HEPC3 (Bailey et al., 2017)	C	E2 AR3	T425, L427, N428, W437, A499, D520, G530	HCVcc	1a, 1b, 2-6	1a, 1b, 2a, 4a, 5a, 6a	16/19	High	CD81 binding	bNAb
HEPC13 (Bailey et al., 2017)	C	E2 AR3	T425, L427, N428, W437, A499, D520, G530	HCVcc	1a, 1b, 2-6	1a, 1b, 2a, 4a, 5a, 6a	16/19	High	CD81 binding	bNAb
HEPC74 (Bailey et al., 2017; Mankowski et al., 2018)	C	E2 AR3	N428, G530, D535	HCVcc	1a, 1b, 2-6	1a, 1b, 2a, 4a, 5a, 6a	17/19	Variable	CD81 binding	bNAb
HEPC43 (Bailey et al., 2017)	C	E2 AR3	T425, L427, N428, G436, W437, L438, G440, F442, Y443, A499, G517, T519,	HCVcc	1a, 1b, 2-6	1a, 1b, 2a, 4a, 5a, 6a	17/19	Variable	CD81 binding	bNAb

			D520, Y527, W529, G530, D535, W616							
IGH505[c] (Meunier et al., 2008; Wahid and Dubuisson, 2013)	NC	E1 stem	H316, W320, M323	HCVpp	1a, 1b, 2-6	1a, 1b, 2a, 4a, 5a, 6a	N/D	High (excl. weak 2a)	Post-binding	bNAb
IGH526 (Meunier et al., 2008; Wahid and Dubuisson, 2013)	NC	E1 stem	H316, A319, W320, M323, M324	HCVpp	1a, 1b, 2-6	1a, 1b, 2a, 4a, 5a, 6a	N/D	Variable	Post-binding	bNAb
Neutralizing MAbs										
e301 (Burioni et al., 2002; Castelli et al., 2017)	C	E2 AR3	T425, L427, N428, W437, L438, G440, F442, D520, W529, G530, D535, W616	VSV/ HCV	1a, 1b	1a, 1b	N/D	Moderate	CD81 binding	NAb
e509 (Castelli et al., 2017; Sautto et al., 2012)	C	E2 AR3	T425, L427, N428, G436-L438, G440, F442, W529, G530, D535, Y594, W616, C620	HCVpp	1a, 1b, 2a	1a	N/D	High	CD81 binding	NAb
H77.16 (Keck et al., 2016; Sabo et al., 2011)	NC	E2 HVR1	P405-K408, N410-I411	FFU assay	1a, 2a	1a	N/D	High	SR-B1 binding early post-attachment	NAb
H77.28 (Sabo et al., 2011)	NC	E2 AR1	D535, T542-P544	FFU assay	1a, 2a	1a	N/D	Weak	Pre-attachment	NAb
HEPC98 (Bailey et al., 2017; Mankowski et al., 2018)	NC	E2 HVR1	L402, P405, K408	HCVpp panel	1a, 1b	1a, 1b	6/19	Variable	Pre-attachment	NAb
HEPC108 (Colbert et al., 2019)	C	E2 AR1	R543, P545, N548, Y594, G635	HCVpp panel	1a, 1b	1a, 1b	15/19	Variable	Unknown	NAb
HEPC111 (Colbert et al., 2019)	C	E2 stem	W672, L666	HCVpp panel	1a, 1b	1a, 1b	14/19	Variable	Pre-/post-attachment	NAb
HEPC112 (Colbert et al., 2019)	C	E1	R259, R260	HCVpp panel	1a, 1b	1a	7/19	Variable	Unknown	NAb
HEPC122 (Colbert et al., 2019)	C	E2 AR3	I422, L441, H445, Y527, W529, W616, C620	HCVpp panel	1a, 1b	1a, 1b	13/19	High/ variable	CD81 binding	NAb
HEPC146 (Colbert et al., 2019)	C	E2 AR3	G517, T534, W529	HCVpp panel	1a, 1b	1a, 1b	16/19	High/ variable	Pre-/post-attachment	NAb
HEPC 151-1 (Colbert et al., 2019)	C	E2 AR3	W529	HCVpp panel	1a, 1b	1a, 1b	16/19	High	CD81 binding	NAb
HEPC 151-2 (Colbert et al., 2019)	C	E2 AR1	R543-P545, N548, Y594, G635	HCVpp panel	1a, 1b	1a	9/19	Variable	Unknown	NAb
HEPC153 (Colbert et al., 2019)	C	E2 AR3	N423, L427, N430, S432, W437, L441, F442, R455, G517, Y527, W529, W616	HCVpp panel	1a, 1b	1a, 1b	17/19	High	CD81 binding	NAb
HEPC154 (Colbert et al., 2019)	C	E2 AR3	N430, S432, W437, F442	HCVpp panel	1a, 1b	1a, 1b	14/19	High/ variable	CD81 binding	NAb
HEPC158 (Colbert et al., 2019)	C	E2 AR1	R543, P544, P545, N548, Y594, G635	HCVpp panel	1a, 1b	1a	9/19	Variable	Unknown	NAb

Non-neutralizing MAbs										
AR1A (Law et al., 2008)	C	E2 AR1	T519, T542, P544, P545, G547, N548, Y632	HCVpp, HCVcc	1a, 1b, 2a, 2b, 4, 5	N/D	N/D	N/A	N/A	Non-NAb
AR1B (Ball et al., 2014; Law et al., 2008)	C	E2 AR1	P544, P545, G547, N548	HCVpp, HCVcc	1a, 1b, 2a, 2b, 4, 5	N/D	N/D	N/A	N/A	Non-NAb
e8 (Castelli et al., 2014)	C	E2 AR1	T542, P544, P545, G547, N548, V633	VSV/ HCV	1b	N/D	N/D	N/A	N/A	Non-NAb
e10 (Bugli et al., 2001; Castelli et al., 2014)	C	E2 AR3	T425, P498, A499, T519, D520, R521, G530	Unknown	1a	N/D	N/D	N/A	N/A	Non-NAb
H60 (Castelli et al., 2014)	C	E2 AR1, HVR2	G470, I472, Y474, R543, P544, P545, Y594	Unknown	1a	N/D	N/D	N/A	N/A	Non-NAb
H77.47 (Sabo et al., 2011)	C	E2 AR1	R543-L546	FFU assay	1a, 2a	N/D	N/D	N/A	N/A	Non-NAb
HEPC50 (Bailey et al., 2017)	C	E2 AR1	R543, P544, P545, G547, Y594, C597, G598, Y632	HCVpp panel	1a, 1b	N/D	0/19	N/A	N/A	Non-NAb
HEPC46 (Bailey et al., 2017; Mankowski et al., 2018)	C	E2 AR1	N541, T542, R543, P544, P545, L546, G547, N548, Y594, C597, G598, V633	HCVpp panel	1a, 1b	N/D	0/19	N/A	N/A	Non-NAb
S1 (Giang et al., 2012)		E2 stem	S512, Y632	N/D	N/A	N/A	N/A	N/A	N/A	E2 stem
AB (Giang et al., 2012)	C	E2 stem	R630, E641	N/D	N/A	N/A	N/A	N/A	N/A	E2 stem
HCVE1-C1 (this manuscript)	C	E1 stem	G311, H312	HCVpp	1a	N/D	N/D	N/A	N/A	Non-NAb

[a]For 1a/1b panel tests, "fraction of 1a strains neutralized" refers to the number of strains of genotype 1a HCVpp that were neutralized by the MAb compared to all tested strains.
In the original characterization of MAbs from (Colbert et al., 2019) the same data used here to identify critical binding residues were analyzed more broadly to recognize binding residues and also residues that might be important for the local structure of the MAb binding site.
Abbreviations: AR1-5, antigenic regions 1-5; cNTR, conserved N-terminal region; C, conformational; NC, non-conformational; NTR, N-terminal region; N/D, not determined; N/A, not applicable.

Table S2. Critical residues for E1E2 structure and function. Related to Figures 2, 3.

Stage	Protein	Region[a]	Critical Residues
Folding	E1	Unknown	D218, I220, H222, G225, *C226*, V227, P228, *C229*, V230, R231, E232, G233, *C238*, W239, V242, V246, T248, D250, G251, L253, P254, L258, D263, R297, H298, T300, Q302, *C304*, N305*, *C306*, H352, G354, F378, D382
Folding	E1	N-terminus	*C207*, N209*, I212, E215
Folding	E1	Putative IFL	D279
Folding	E2	Front layer	T425*, N428, *C429*
Folding	E2	β-sandwich	*C494*, I496, V497, A499, V502, *C503*, G504, P505, V506, Y507, *C508*, F509, P511, V514, V516, T518, F537, N540*, F550, G551, *C552*, W554, M555, N556*, G559, T561, *C564*, G565, *C569*
Folding	E2	CD81BL	D520, G523, G530, D535
Folding	E2	Back layer	G600, W602, I603, *C607*, M608, V609, D610, Y611, R614, H617, Y618, P619, T621, N623*, T625*, H638, L640, A642, A643, *C644*
E1E2 dimer assembly	E1	N-terminus	Y201, T204, N205, D206
E1E2 dimer assembly	E2	Unknown	*C581*, *C585*
E1E2 dimer assembly	E2	Back layer	*C459*, *C486*
E1E2 dimer assembly	E2	β-sandwich	W487, R543, P544, P545, W549
E1E2 dimer assembly	E2	Stem	*C652*, R657, D658, *C677*, F679, L692, D698, Q700
CD81 LEL binding	E2	cNTR	W420
CD81 LEL binding	E2	Front layer	I422, S424, L427, N430*, S432*, G436, W437, L438, G440, L441, F442, Y443
CD81 LEL binding	E2	β-sandwich	V515
CD81 LEL binding	E2	CD81BL	T519, T526, Y527, W529
CD81 LEL binding	E2	Back layer	W616
Infectivity	E1	E1 cleavage	Y192, Q193
Infectivity	E1	Unknown	R259, H261, T292, P310, G311, I313
Infectivity	E1	Putative IFL	S273, Y276, G282, F285, L286
Infectivity	E1	Stem	M318, W320, M322, M323, W326, P328, A330, A331, L332, V333, V334, L337, R339, M347
Infectivity	E1	TM	V355, F362, M364, N367, L377
Infectivity	E2	HVR1, SR-B1	H386, G390, G398, L399, N410, I411
Infectivity	E2	cNTR	Q412, I414, T416
Infectivity	E2	Front layer	K446, F447, S449, G451, L456
Infectivity	E2	VR2	R460, G470, R483
Infectivity	E2	Back layer	Y489, P490, T593, R596, W646, R648, G649
Infectivity	E2	β-sandwich	R492, N541, L546
Infectivity	E2	CD81BL	R521, T534*
Infectivity	E2	igVR	G572, L579
Infectivity	E2	Stem	L662, S663, L665, L666, L667, S668, T669, T670, Q671, W672, L675, T681, L682, L685, S686, T687, G688, H691, H693, I696, V697, Y701, L702, Y703, V705, S707, I709, A710, I714, K715
Infectivity	E2	TM	L722, L735, W736, Q743

Critical cysteines are italicized; * residues in glycosylation sequences are denoted by asterisks. Abbreviations: CD81BL, CD81 binding loop; cNTR, conserved N-terminal domain; HVR1, hypervariable region 1; igVR, intergenotypic variable region; IFL, internal fusion loop; LEL, long extracellular loop; SR-B1, scavenger receptor B type 1 binding site; TM, transmembrane domain.

[a]Regions of the HCV Env sequence or structure were identified from the literature and reflect the current consensus based on all available experimental evidence (Akbar and Jusoh, 2013; Drummer et al., 2006; Drummer et al., 2007; Flint et al., 1999; Lavillette et al., 2007; Tong et al., 2017).