SUPPLEMENTAL FIGURES

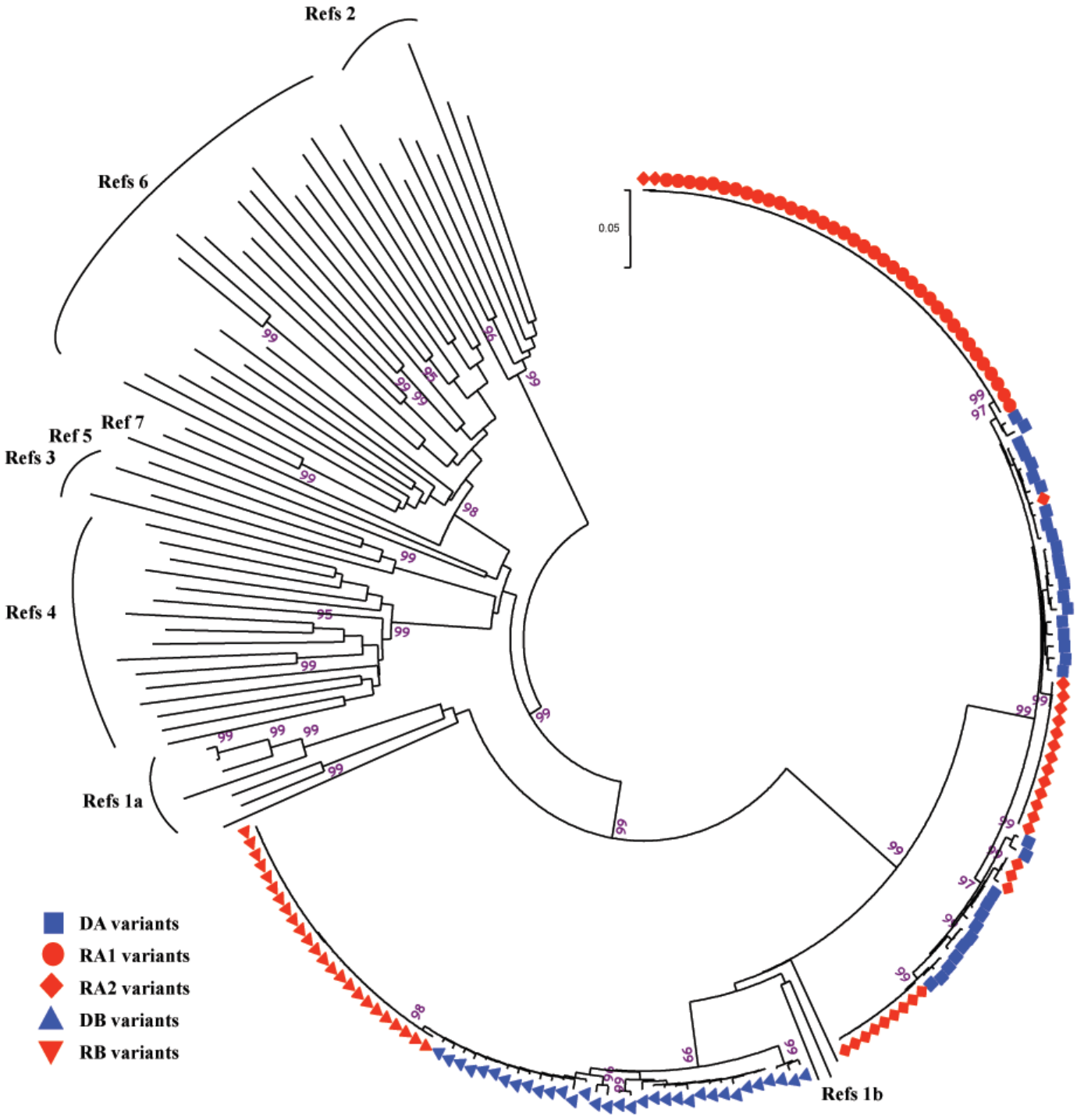


Figure S1. Combined phylogenetic analysis of donor and recipient virus populations. A neighbor-joining tree constructed with all E1E2 nucleotide sequences available from donor DA (blue square), recipient RA1 (red circle), recipient RA2 (red diamond), donor DB (blue triangle) and recipient RB (red triangle) along with HCV genotype 1 to 7 reference sequences (Los Alamos National Laboratories HCV database) is shown. Only bootstrap values ≥ 95% are presented. The scale bar corresponds to 0.05 nucleotide substitutions per site.

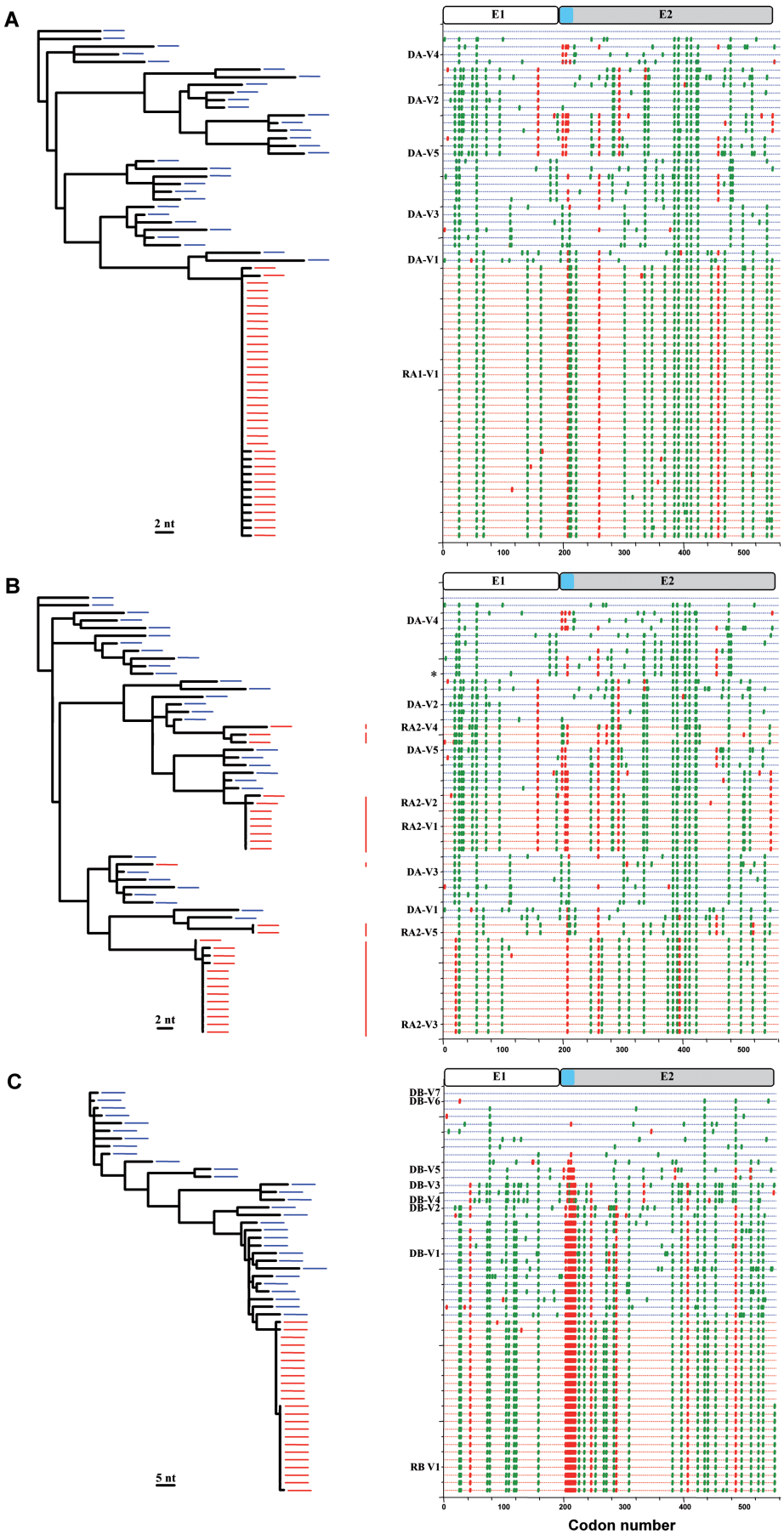

A
E1
E2
DA-V4
DA-V2
DA-V5
DA-V3
DA-V1
RA1-V1
2 nt
0
100
200
300
400
500
B
E1
E2
DA-V4
*
DA-V2
RA2-V4
DA-V5
RA2-V2
RA2-V1
DA-V3
DA-V1
RA2-V5
RA2-V3
2 nt
0
100
200
300
400
500
C
E1
E2
DB-V7
DB-V6
DB-V5
DB-V3
DB-V4
DB-V2
DB-V1
RB V1
5 nt
0
100
200
300
400
500
Codon number

Figure S2. Phylogenetic relationships and patterns of substitution in HCV E1E2 nucleotide sequences between the transmission pair variants. E1E2 nucleotide sequences derived from recipient RA1 **(A)**, RA2 **(B)** and RB **(C)** (red) were analyzed by neighbor-joining trees (left panels) and Highlighter plots (right panels), with pretransmission donor sequences included (blue). Sequences with stop codons or deletions altering the open reading frame are not shown. Nucleotide polymorphisms are indicated by a colored tick mark (green bars: synonymous substitutions, red bars: non synonymous substitutions). The scale bar represents two (A, B) or five nucleotides (C). A schematic diagram of the E1E2 sequence, showing the location of hypervariable region 1 (HVR1) of E2 in light blue, is provided above the Highlighter plots.

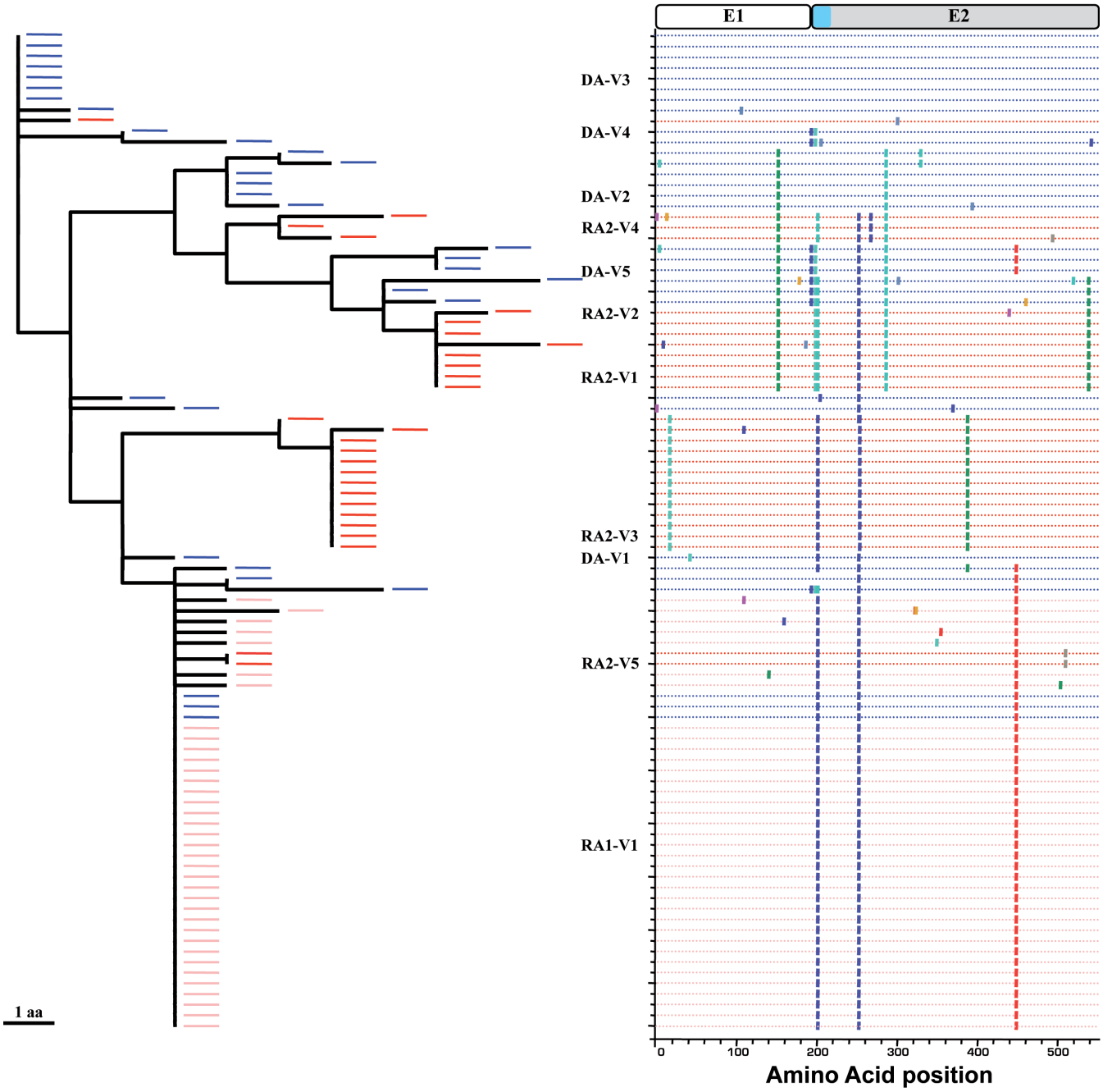
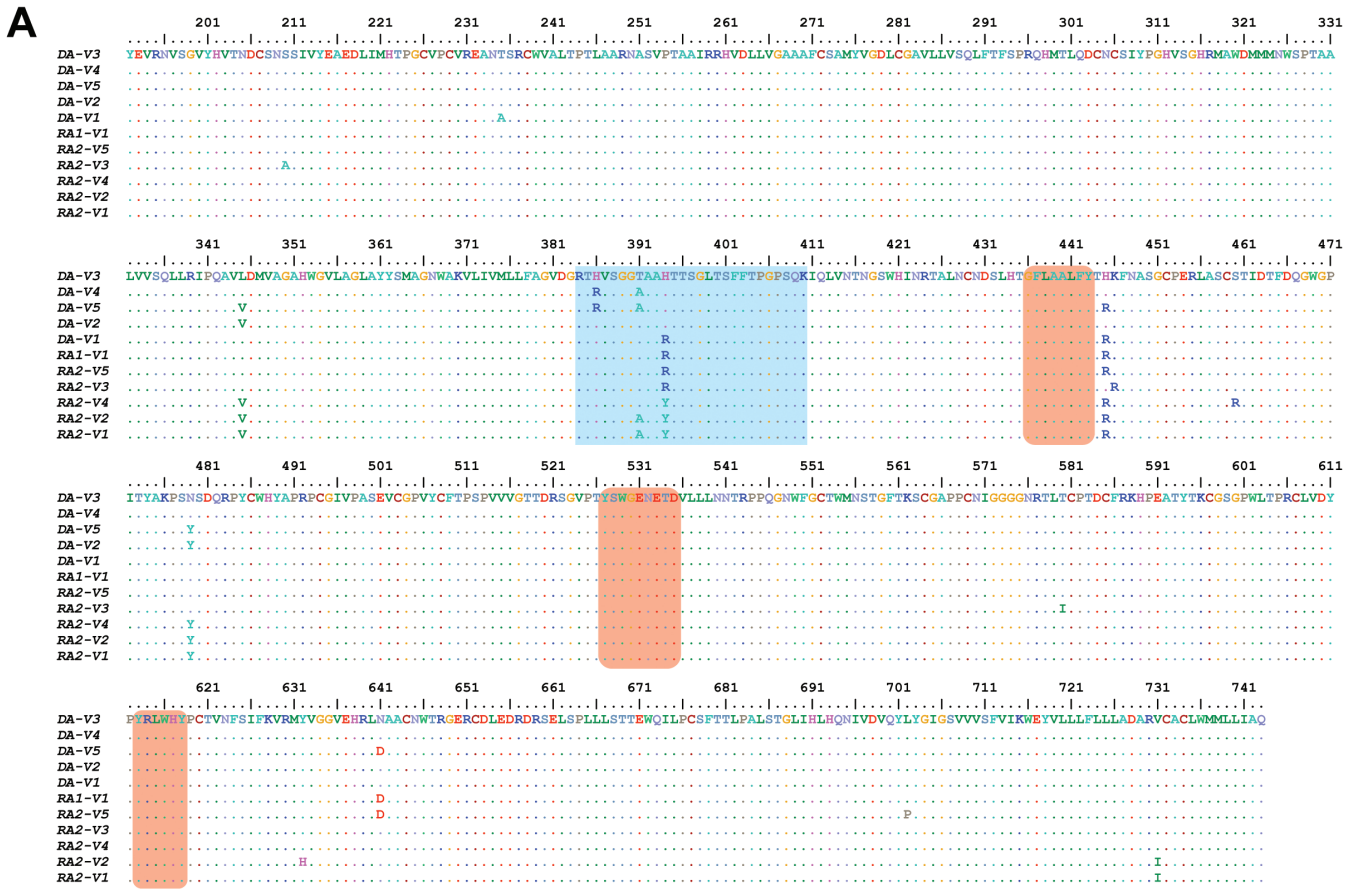


Figure S3. Phylogenetic relationships and patterns of substitution in HCV E1E2 amino acid sequences between the transmission pairs DA/RA1/RA2. E1E2 amino-acid sequences derived from recipient RA1 (dark red) and RA2 (light red) were analyzed by neighbor-joining tree (left panel) and Highlighter plot (right panel), with pretransmission donor DA sequences included (blue). Polymorphisms are indicated by a colored tick mark specific for each amino acid, according to the color scheme of BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html). A schematic diagram of the E1E2 proteins, showing the location of HVR1 of E2 in light blue, is provided above the Highlighter plot. The scale bar represents one amino-acid.


A
DA-V3
DA-V4
DA-V5
DA-V2
DA-V1
RA1-V1
RA2-V5
RA2-V3
RA2-V4
RA2-V2
RA2-V1

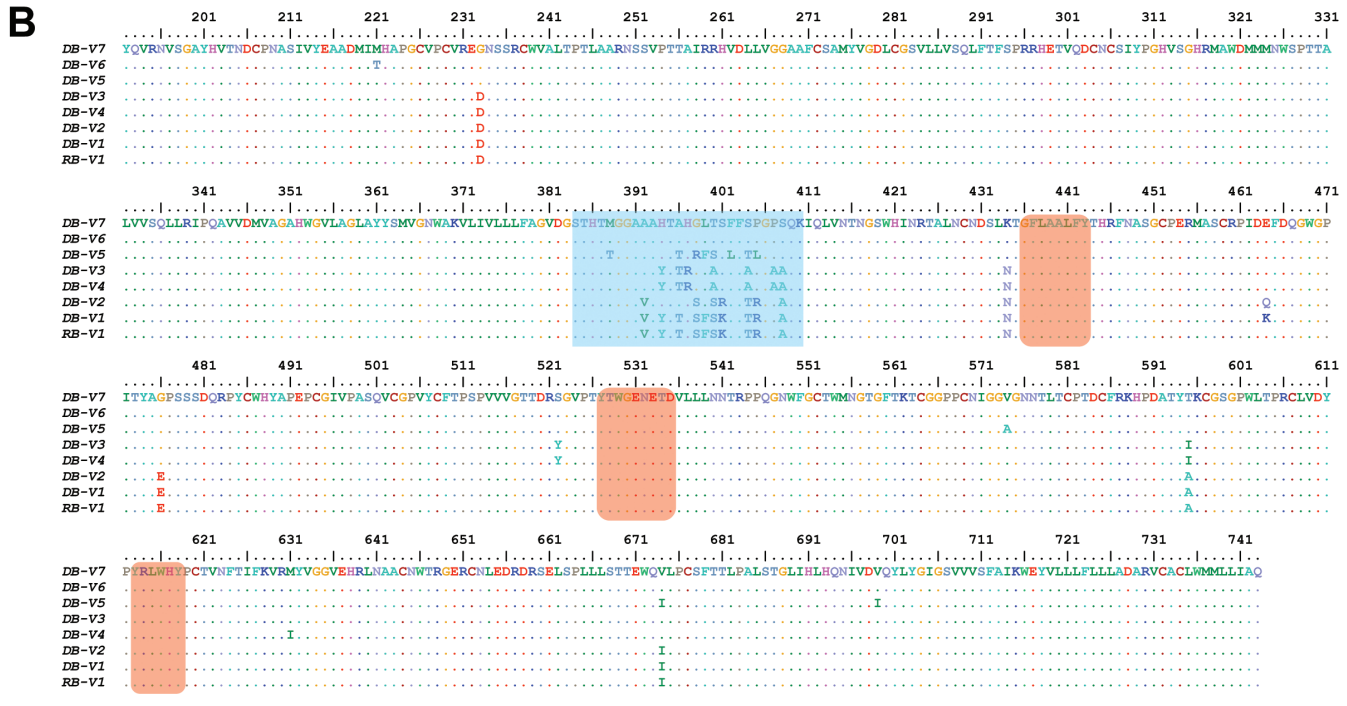

B
DB-V7
DB-V6
DB-V5
DB-V3
DB-V4
DB-V2
DB-V1
RB-V1

Figure S4. Comparative alignment of amino-acid sequences of E1E2 envelope glycoprotein variants selected for phenotypic analyses. (A) Amino-acid sequences of E1E2 variants derived from donor DA and recipients RA1/RA2. **(B)** Amino-acid sequences of E1E2 variants derived from donor DB and recipient RB. The sequences are presented in the same order as in Fig. 2. Dots indicate amino-acid identity. Mutations are indicated in the amino acid one-letter code. Amino-acid sequences encompassing HVR1 (highlighted in blue) and CD81 binding domains (highlighted in red) are highlighted.

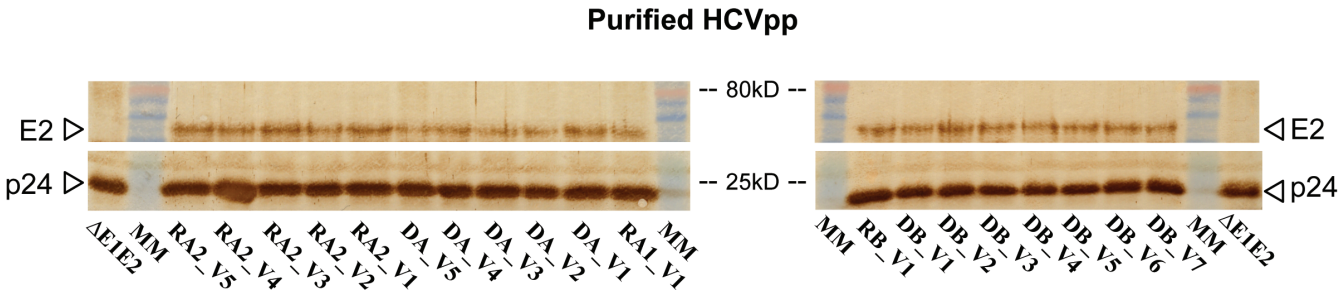


Figure S5. Western blot analysis of envelope glycoprotein incorporation into HCVpp produced by 293T cells. The upper panel shows the blot of E2 glycoproteins derived from the variants selected for phenotypic analyses. These E2 glycoproteins were detected with the mAb 3/11 (see Materials and Methods). The blot was stripped and reprobed with a specific rabbit antiserum against HIV-1 p24 (lower panel). The positions of E2 and p24 are shown on the side of the panels. MM, molecular markers.