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Supplementary Materials for

Global and local envelope protein dynamics of hepatitis C virus determine broad antibody sensitivity

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This PDF file includes:

Figs. S1 to S6

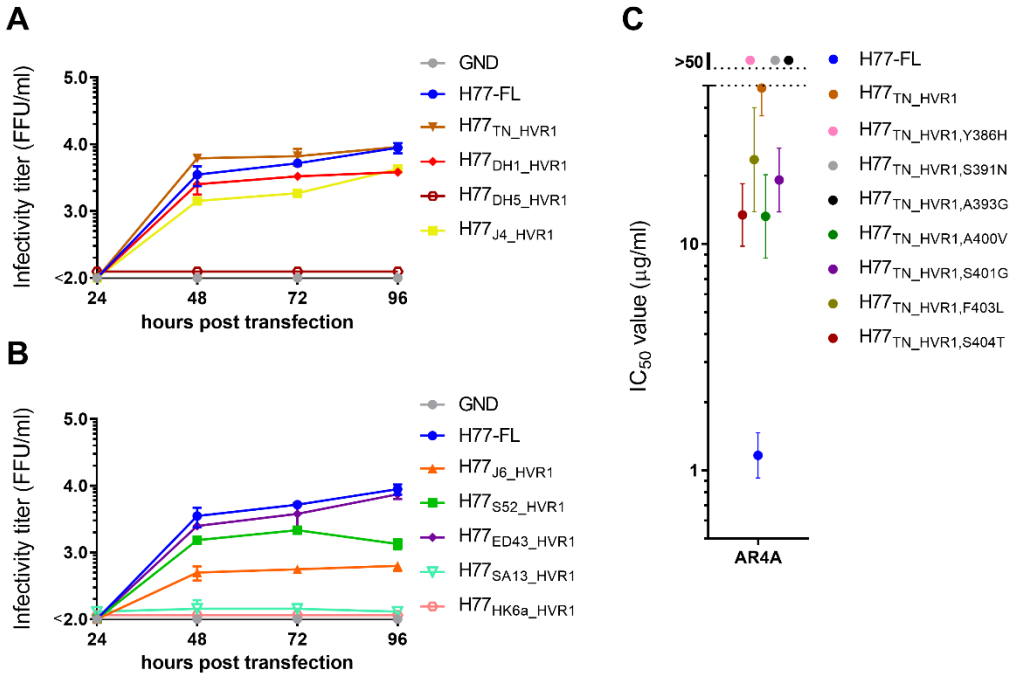

Figure S1. HCVcc infectivity and broad neutralization sensitivity was affected by polymorphisms in HVR1. (A-B) In vitro transcribed RNA of H77/JFH1-based recombinants with HVR1 from H77 (1a), TN (1a), DH1 (1b), DH5 (1b), J4 (1b), J6 (2a), S52 (3a), ED43 (4a), SA13 (5a) and HK6a (6a) was used to transfect Huh7.5 cells and infectivity titers were determined in collected supernatants 24, 48, 72, and 96 hours post-transfection. Values represent the mean of triplicate titer determinations and error bars represent standard deviation (SD). GND, HCV replication deficient control. **(C)** Neutralization by the monoclonal bNAb AR4A of H77-based recombinants for which H77-HVR1 polymorphisms were introduced singly into $H77_{TN\_HVR1}$. The data was analyzed using three parameter dose-response regression to calculate IC_{50} values and 95% confidence intervals (Graphpad PRISM 8.0.0).

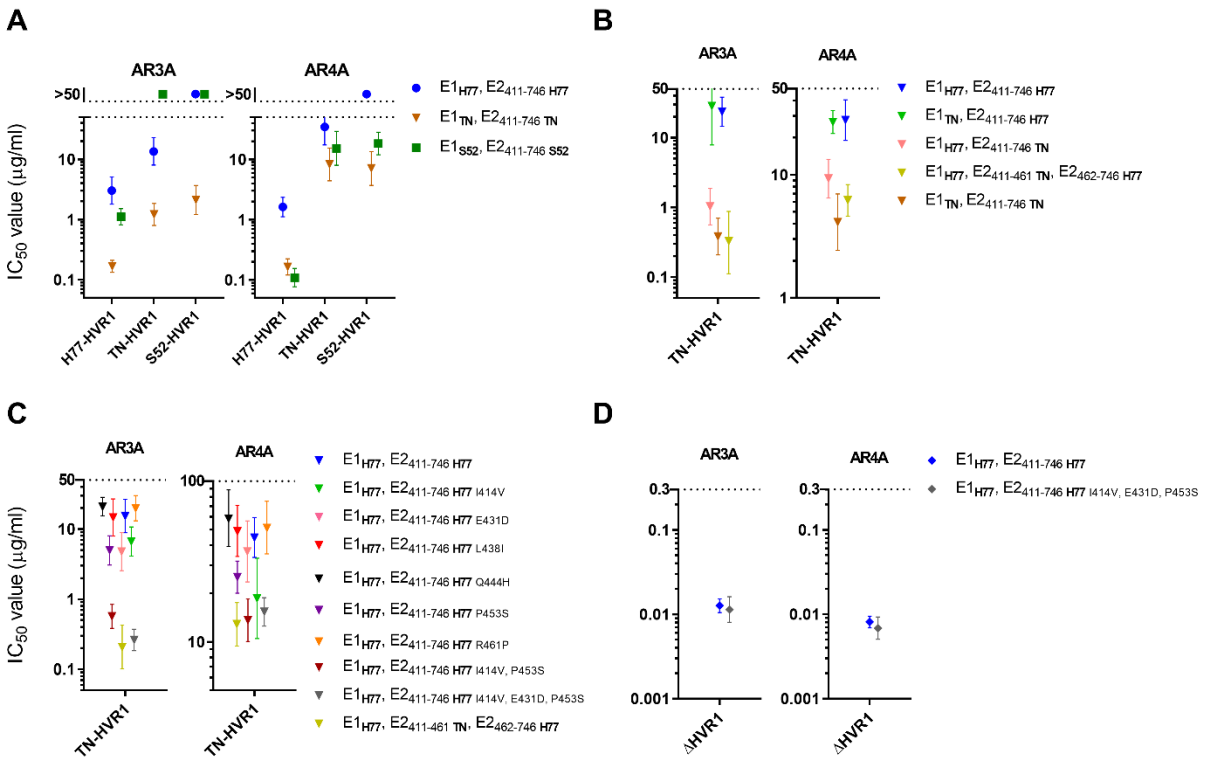

Figure S2. Polymorphisms at positions 414, 431 and 453 outside of HVR1 also determined the level of HVR1-mediated bNAb sensitivity but in an HVR1-dependent manner. Neutralization by the monoclonal bNAbs AR3A and AR4A of **(A)** HVR1-swapped JFH1-based recombinants of H77, TN and S52, **(B)** HVR1-, E1-, $E2_{411-746}$- or $E2_{411-461}$ swapped JFH1-based recombinants of H77 or TN, **(C)** JFH1-based recombinants of $H77_{TN-HVR1}$ and with TN polymorphisms introduced at the indicated positions either singly or in selected combinations, **(D)** JFH1-based recombinants of $H77_{\Delta HVR1}$ with and without TN polymorphisms introduced at the indicated positions. $E1_{H77}E2_{411-461\ H77}$ with H77-HVR1 (A), TN-HVR1 (A-C) and S52-HVR1 (A) are referred to as H77-FL, $H77_{TN\_HVR1}$ and $H77_{S52\_HVR1}$, respectively, in the other figures. The data was analyzed using three parameter dose-response regression to calculate IC_{50} values and 95% confidence intervals (Graphpad PRISM 8.0.0).

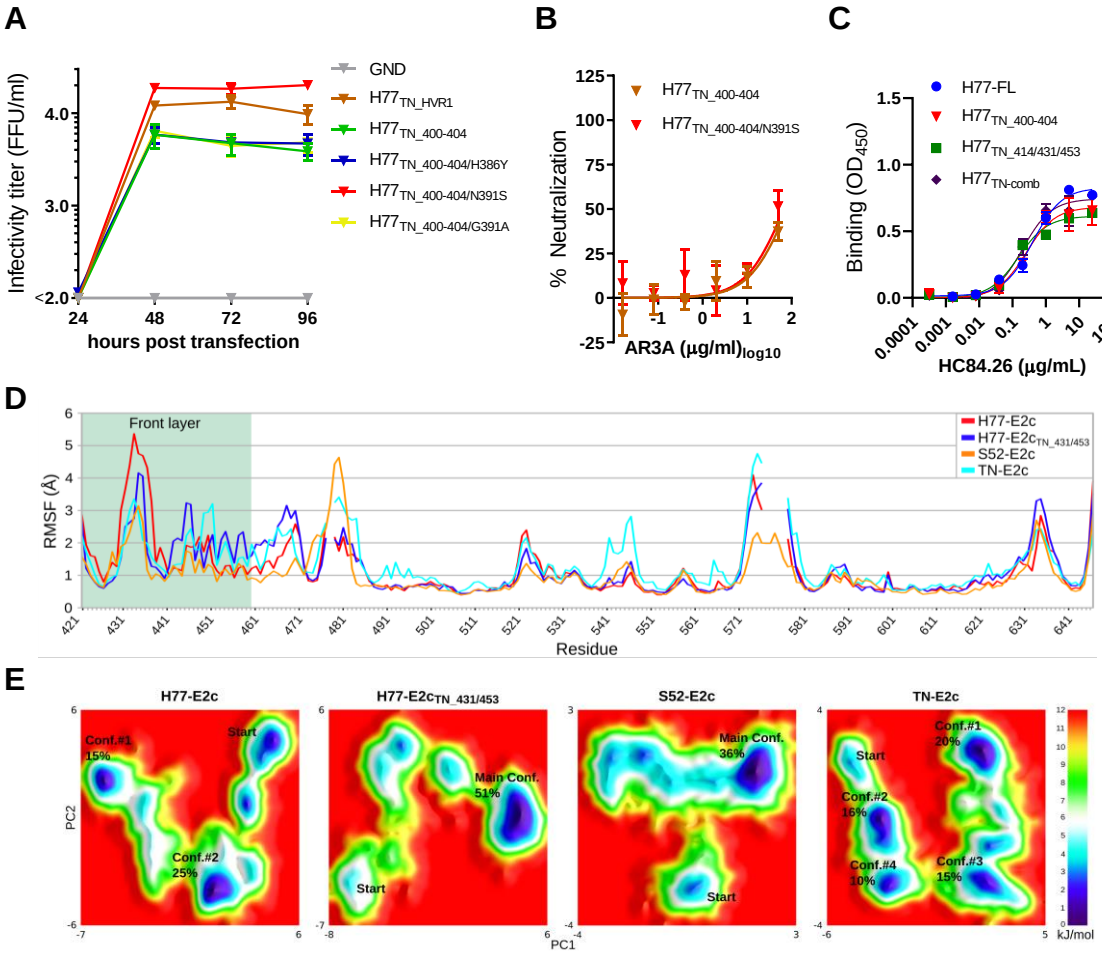

Figure S3. Protective envelope polymorphisms at positions 400-404 in HVR1 or outside HVR1 at position 414, 431 or 453 shifted global envelope conformation dynamics. (**A**) In vitro transcribed RNA of the indicated H77 recombinants was used to transfect Huh7.5 cells and infectivity titers were determined from supernatants collected from transfected cultures. Values represent the mean of triplicate titer determinations and error bars represent (SD). GND, HCV replication deficient control. (**B**) Neutralization of indicated H77 recombinants with NAb AR3A. The data was analyzed using three parameter dose-response regression (Graphpad PRISM 8.0.0). (**C**) Binding of HC84.26 to sE2 (positions 384-645, see Fig. 1A) in ELISA. Values are means of duplicates ± SD. (**D**) Peptide backbone residue flexibility (root mean square fluctuation (RMSF)) was calculated for all alpha carbons along 500 ns molecular dynamics (MD) simulations of H77-E2c, H77TN_431/453-E2c, TN-E2c and S52-E2c. E2 front layer (positions 421-460) is highlighted in color. (**E**) Gibbs free energy landscape plot of H77-E2c, H77-E2c TN_431/453, TN-E2c and S52-E2c based on PCA. The graphs show how, after the initial equilibration phase ("Start" point), E2c explores distinct conformations in a sequence-dependent manner. The percentage of time spent in each most represented conformation is reported.

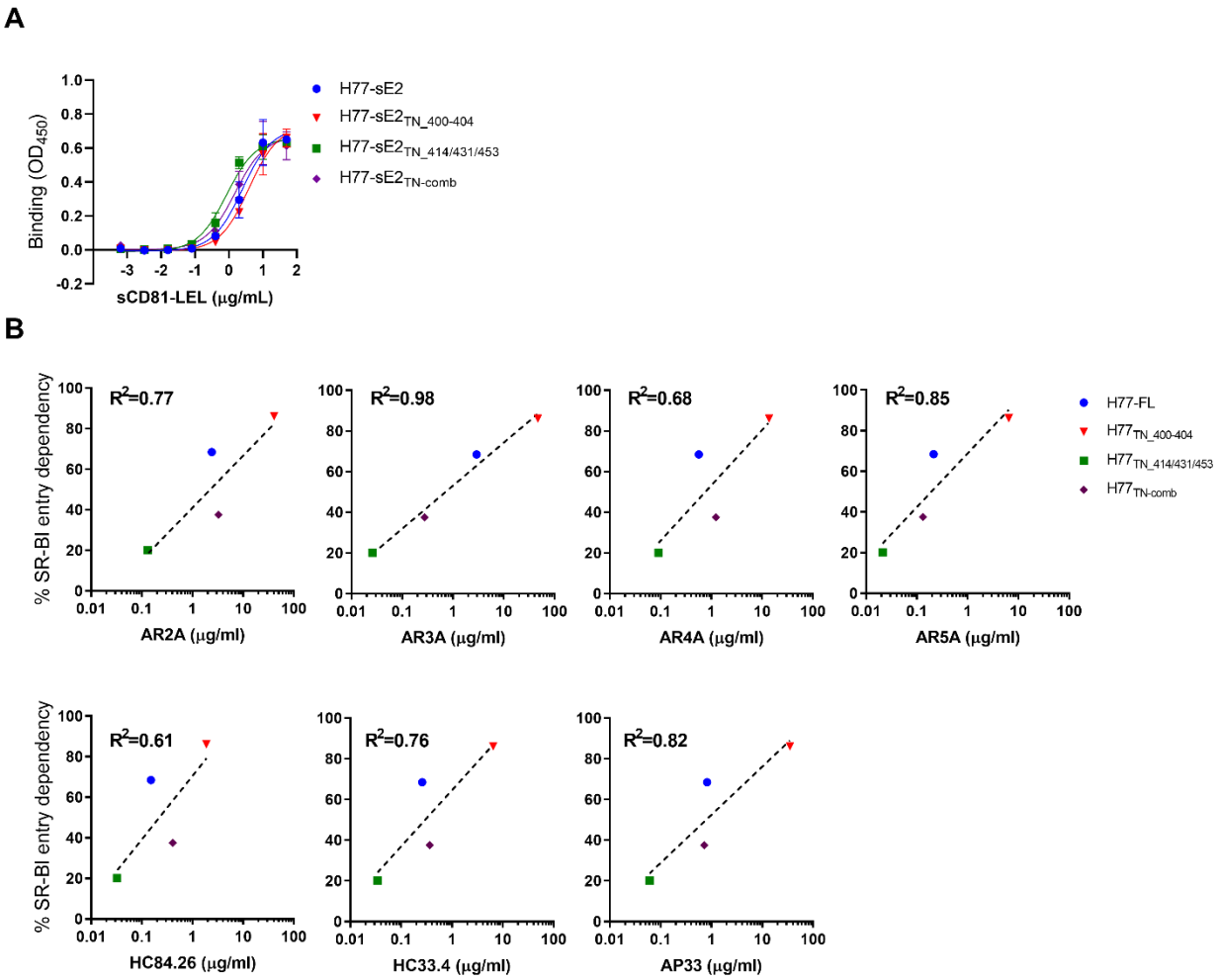

Figure S4. Cross-epitope NAb resistance correlated strongly with SR-BI entry dependency. (**A**) Binding in ELISA to sE2 by sCD81. The assay was performed in duplicate, and values are means ± SD. (**B**) IC_{50} values against AR2A, AR3A, AR4A, AR5A, HC84.26, HC33.4 and AP33 (from Fig. 3A and Fig. 5A) for the indicated H77 recombinants plotted against SR-BI entry dependency calculated as outlined in Fig. 4C (Bmax).

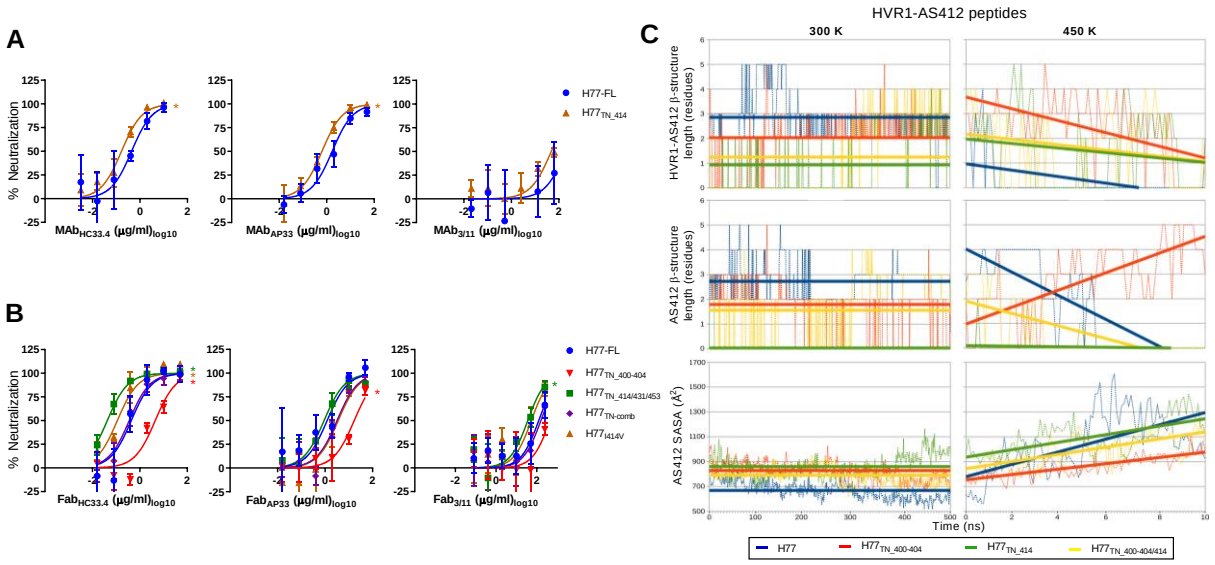

Figure S5. The conformational space of AS412 is skewed towards β-hairpin-like conformations in "closed" HCV E1/E2 states. (**A-B**) The indicated H77 recombinants were subjected to dose-response neutralization assays using dilution series of MAbs or Fabs of HC33.4, AP33 or 3/11 in quadruplicates with 8 wells of virus only. The data was analyzed using three parameter dose-response regression to calculate IC_{50} values and 95% confidence intervals (Graphpad PRISM 8.0.0). All IC_{50} values for H77 recombinants were compared with the unmodified H77-FL in two-tailed t-tests with Welch's correction. Testing was done at the 95% confidence level ($p < 0.05$) and corrected for multiple testing (tested p-value: 0.05 in A, 0.0125 in B). *Neutralization sensitivity of the H77 recombinant with TN-polymorphisms was statistically significantly different from the unmodified H77-FL. (**C**) β-sheet length of HVR1-AS412 (upper panels) or within AS412 (middle panels) and exposure of AS412 (lower panels) during 500ns MD simulation at 300K (left panels) and during 10ns 450K MD (right panels) performed using the most visited 300K structure as a starting point. Due to the stability of all systems at 300K, lines represent the average values. Conversely, in 450K graphs, lines represent the trend, thus highlighting the progressive protein unfolding.

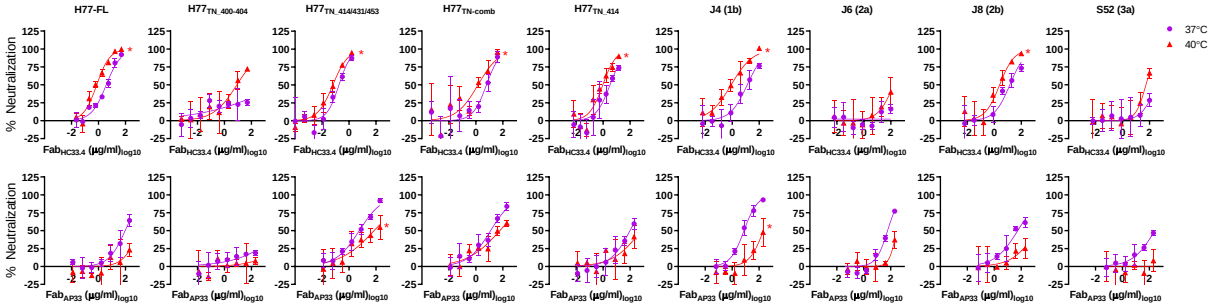

Figure S6. The β-hairpin conformation of AS412 is more prevalent at lower temperatures. Neutralization by HC33.4 or AP33 Fabs of the indicated H77 recombinants and JFH1-based recombinants of J4, J6, J8, S52 at 37°C or 40°C. Virus stocks of the indicated recombinants were subjected to dose-response FFU reduction neutralization assays using dilution series of the Fabs in triplicates with 6 wells of virus only. The data was analyzed using four parameter dose-response regression to calculate IC_{50} values and 95% confidence intervals (Graphpad PRISM 8.0.0), shown in Fig. 5D and E. IC_{50} values were compared in two-tailed t-tests (Graphpad PRISM 8.0.0) with Welch's correction. Testing was done at the 95% confidence level ($p < 0.05$). *Neutralization sensitivity at 40°C was statistically significantly different from the relevant HCV recombinant at 37°C.