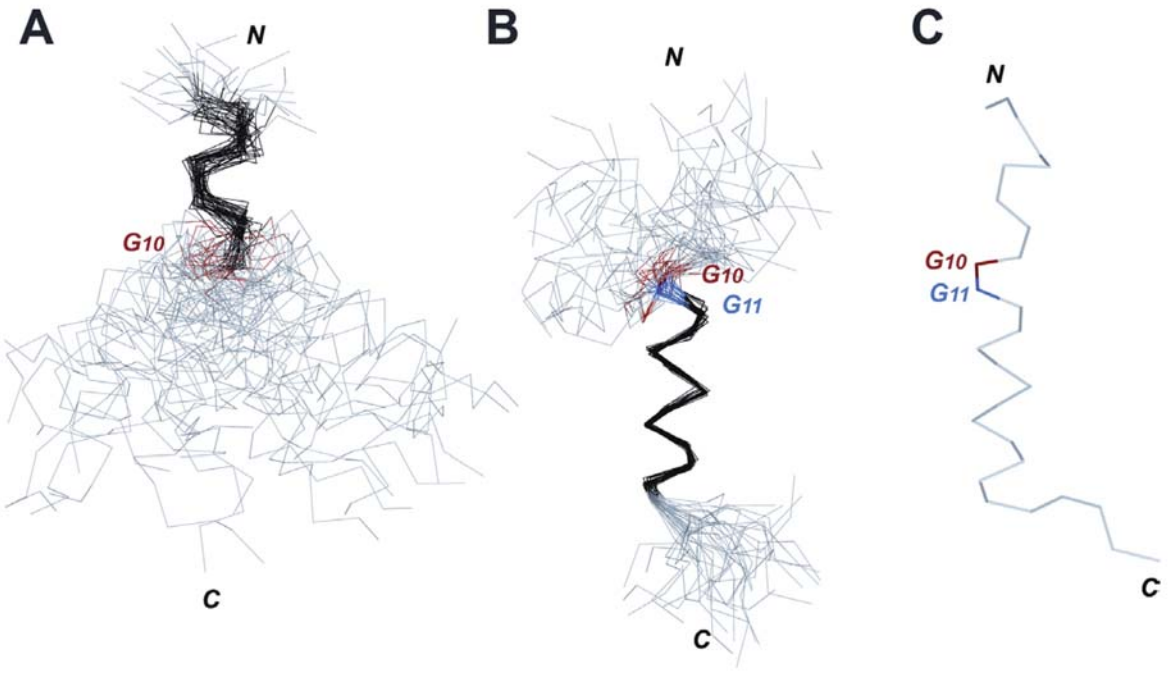
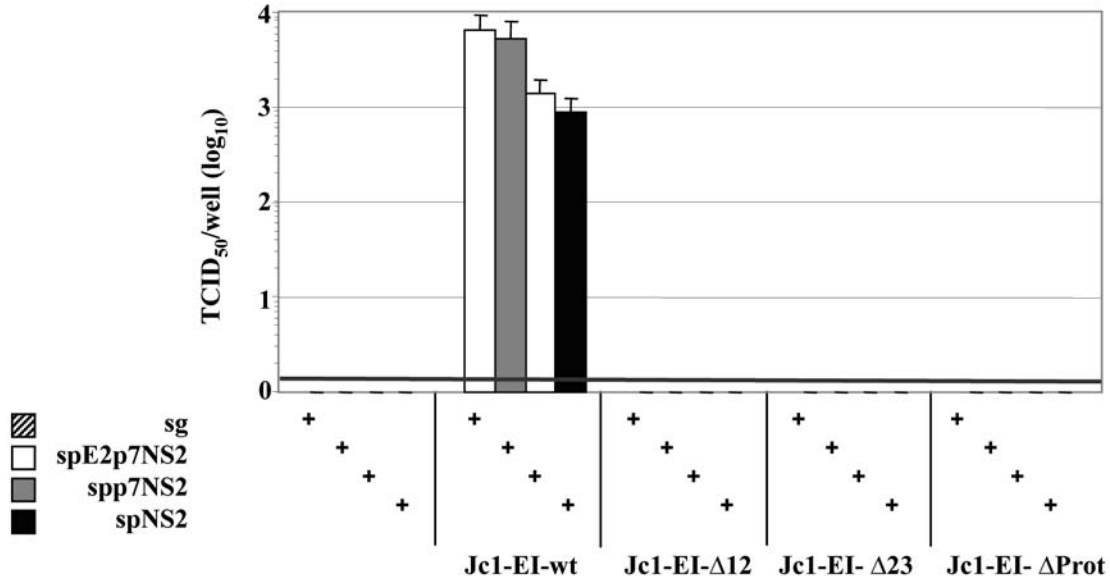


Supplementary Figure 1: Structural characterization of the NS2[1-27] peptide in 50% TFE. (A) and (B), superimposition of the backbone heavy atoms (N, Cα, and C') of the 40 final structures (PDB entry 2JY0) for the best overlap of either residues 3-9 or 12-21, respectively, which correspond to the α-helical segments. (C) Representative structure model selected for its elongated shape that is required to cross the phospholipid bilayer.



Supplementary Figure 2: Exclusion of RNA recombination between NS2 mutant RNA and helper replicon RNA. Huh7.5 cells were transfected with constructs specified in the bottom (for description of the constructs see Fig. 8A). Cell culture medium was harvested after 48 hours and used for infection of Huh7.5 cells. Supernatants of this 2nd round of infection were harvested after 72 hours and utilized for $TCID_{50}$ assay. A representative result of two independent experiments with standard deviations is shown.

Supernatants harvested from cells that had been co-transfected with Jc1-EI-wt and the sg replicon (which lacks NS2) or helper replicon RNAs (encoding E2-p7-NS2 or p7-NS2 or NS2) consistently contained infectious HCV particles. The decline in virus titers attained in the co-transfections with the helper RNAs can be attributed to their cytopathogenicity (see also Fig. 8C). However, with none of the NS2 mutants, infectious virus particles were recovered after second passage. The fact that we obtained infectious particles in the supernatant of cells after primary cotransfection (see Fig. 8B), but not after this secondary passage clearly rules out RNA recombination between the RNA genome of a given NS2 mutant and the helper RNA. Thus, NS2 mutants can be rescued by trans-complementation.