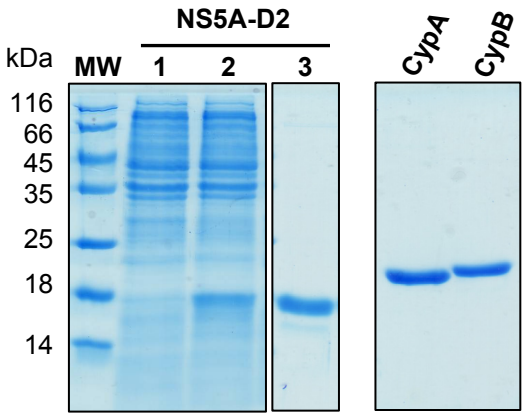
Supplemental Figure 1



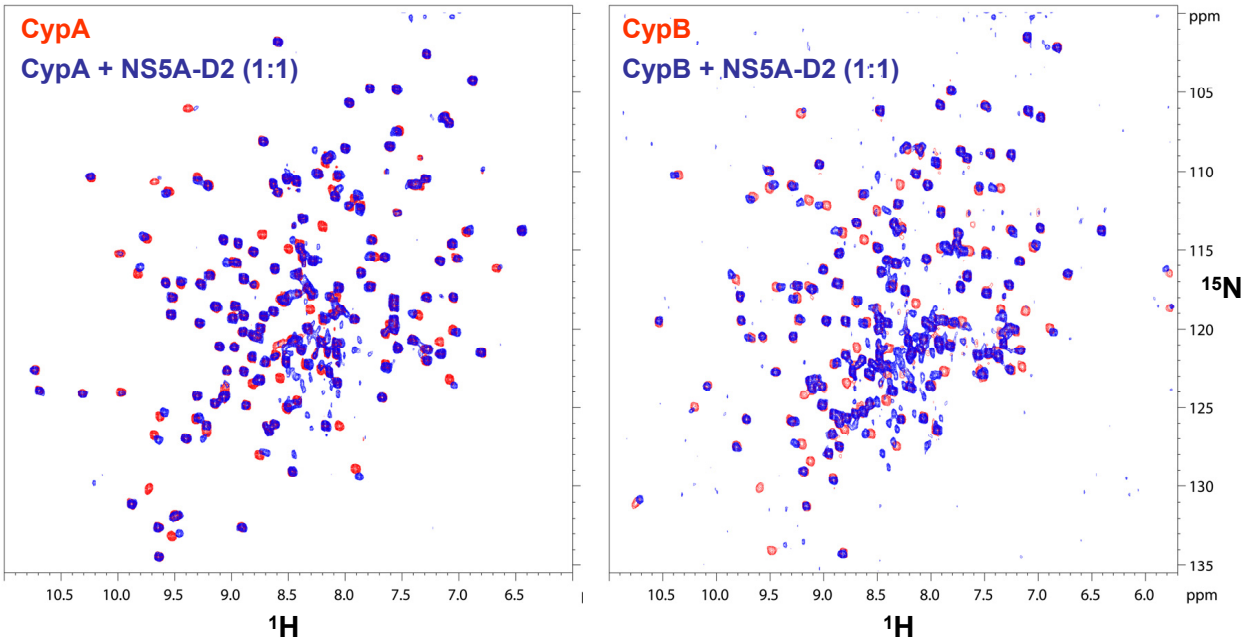
Expression and purification of domain 2 of NS5A and Cyclophilins. Recombinant proteins were analyzed by 15% SDS-PAGE and stained with Coomassie blue. Lane 1, total cell extract of *E. coli* electroporated with pT7-7-NS5A-D2 plasmid before and after, Lane 2, induction of expression. Lane 3, purified recombinant [^{15}N,^{13}C]-labelled NS5A-D2 (11.6kDa). The minor band in the SDS-PAGE is a proteolytic product of the D2 domain that we identified by mass spectroscopy (data not shown). CypA and CypB correspond to the purified recombinants Cyclophilin A (20.1kDa with HisTag) and Cyclophilin B (20.36kDa).

Supplemental Table 1.

NS5A-D2 min.	^{1}HN (ppm)	^{15}N (ppm)	^{13}Cα (ppm)	^{13}Cβ (ppm)	^{13}CO (ppm)	% min.
Q267	7.745	117.930	56.778	29.375	175.651	4.4
T268	8.289	115.570	61.704	70.269	173.558	8.1
E271	8.767	122.759	57.486	30.152	176.642	7.3
R273	8.309	124.049	56.204	30.760	174.869	6.4
V274	7.852	119.408	59.129	34.228	174.567	7.6
V276	8.168	124.467	62.714	32.525	176.289	7.5
L277	8.278	125.881	54.727	42.700	176.664	9.1
L280	8.087	123.768	54.955	42.933	175.787	8.4
E281	7.937	120.648	53.872	30.938	174.592	11.9
M283	8.624	121.590	55.888	33.251	175.883	7.1
L290	8.045	121.861	55.311	42.766	176.438	9.1
S293	8.546	117.165	58.402	63.817	174.356	9.8
I294	8.030	122.238	58.200	40.431	174.873	6.9
S296	8.617	116.570	58.896	64.190	175.089	4.7
E297	8.708	122.768	56.972	29.797	176.438	8.5
L300	7.958	122.093	52.756	43.206	175.522	13.6
R302	8.712	121.534	56.704	30.418	176.588	12.4
A311	8.629	124.146	53.376	18.507	177.463	8.3
W312	7.287	114.668	56.449	28.874	175.829	10.2
A313	7.518	124.740	51.887	19.351	176.622	11.6
D316	8.289	118.686	53.943	40.867	175.548	12.0
W325	7.770	121.081	57.184	29.198	175.840	6.4
R326	7.634	122.381	55.880	30.511	176.660	8.3
D329	8.316	119.212	53.473	40.750	174.477	6.9
Y330	7.836	120.158	57.832	38.815	174.565	5.5
Q331	7.986	124.351	52.965	29.280	172.528	6.5
A339	8.356	126.985	52.332	19.232	176.046	17.0
L340	7.894	120.056	52.725	42.943	175.945	8.2
L342	8.573	121.958	55.685	42.184	177.509	12.7

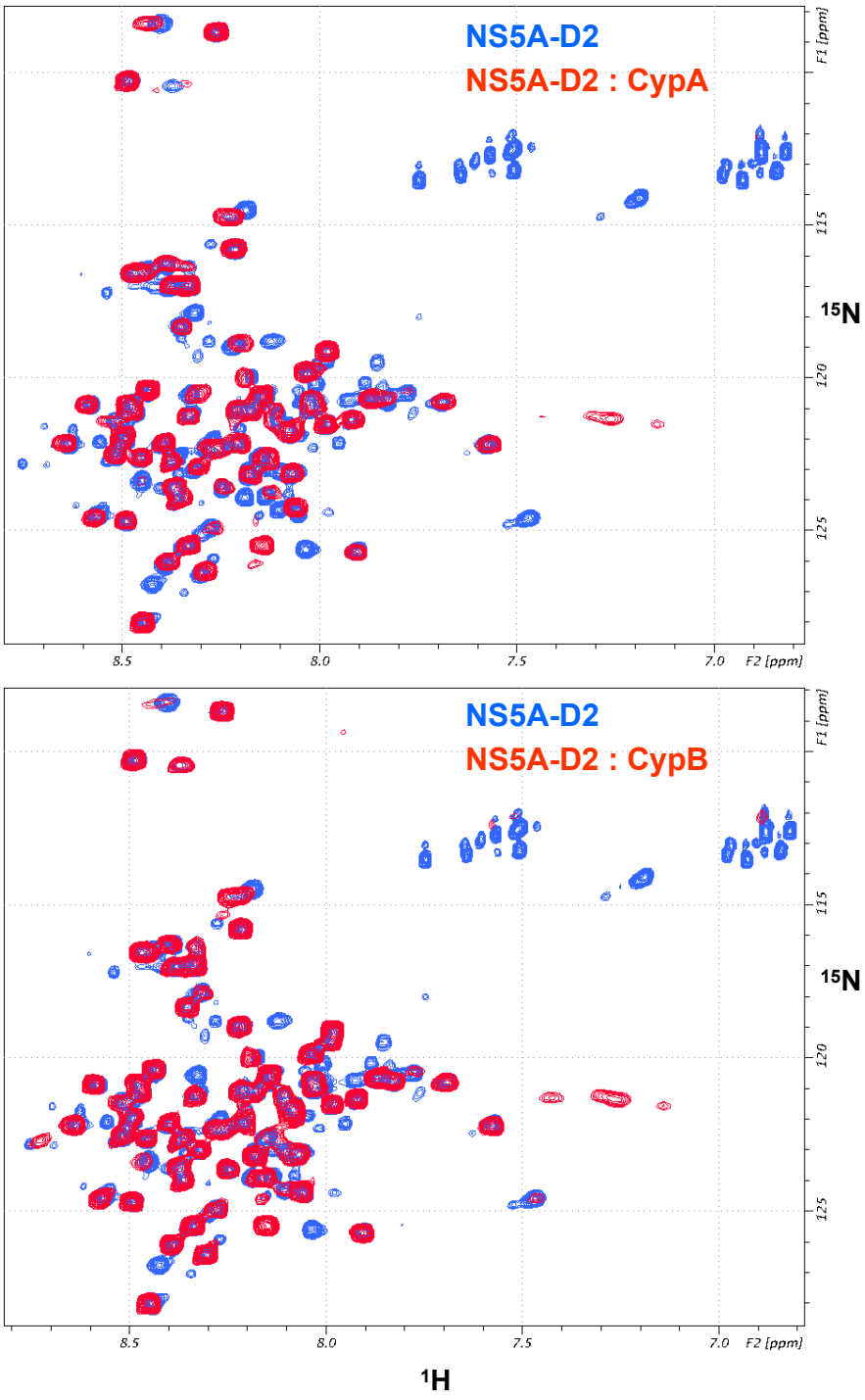
Assignments and quantifications of minor NMR resonances in NS5A-D2 ^{1}H-^{15}N-HSQC spectrum. Because of the low amide proton dispersion in the proton dimension, percentages for minor resonances compared to the corresponding major peaks were measured using maximal peak intensities rather peak integrals.

Supplemental Figure 2



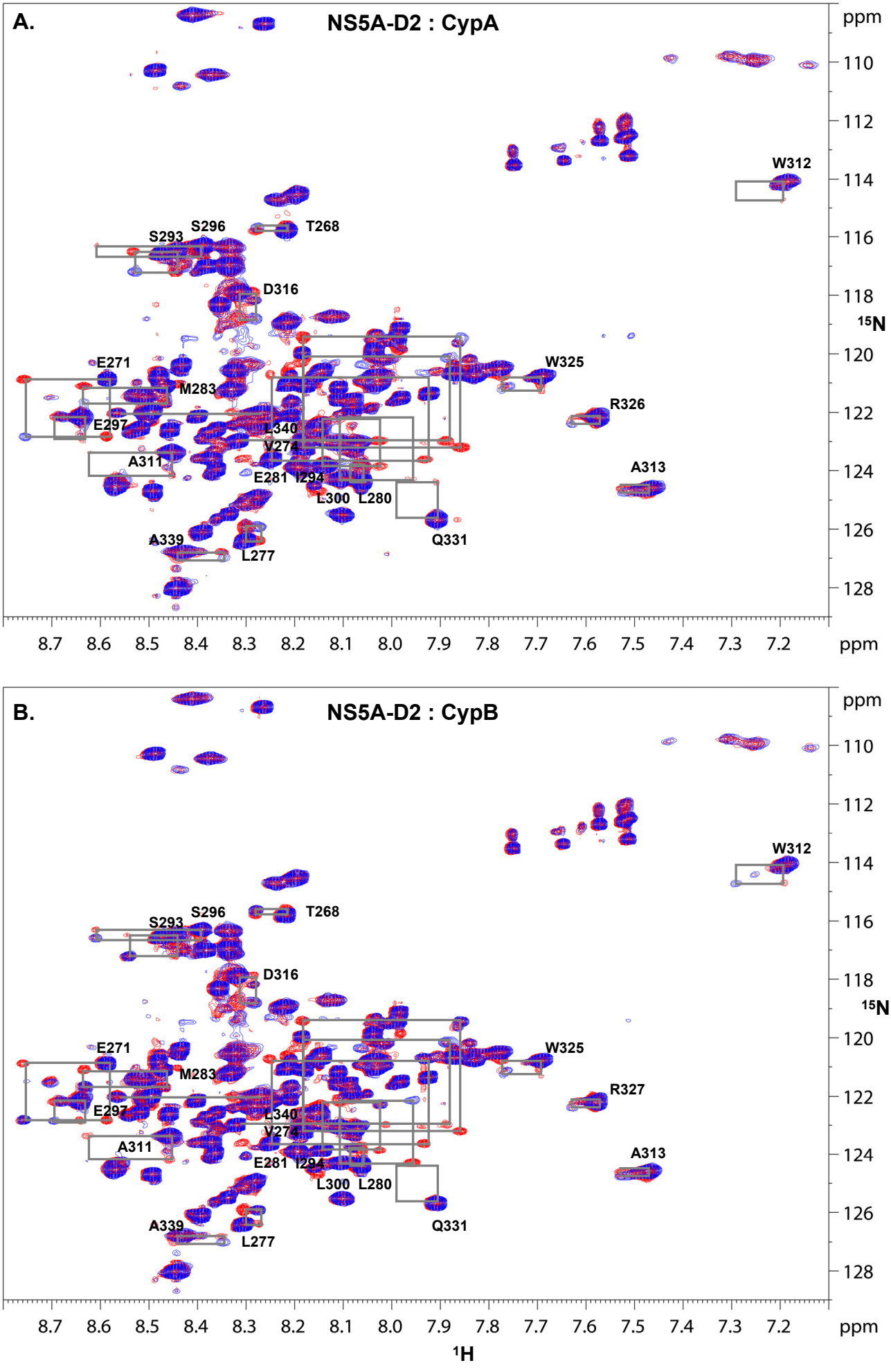
Interaction of human Cyclophilins with NS5A-D2. Each panel corresponds to the superimposition of two [^{1}H,^{15}N]-planes extracted from HNCO spectra. The first one, in red, was acquired on the [^{15}N,^{13}C]-cyclophilin alone, CypA on the left and CypB on the right, whereas the second one, in blue, was acquired on a mixture of [^{15}N,^{13}C]-Cyclophilin and [^{15}N]-NS5A-D2 in a molar ratio of 1:1. Addition of NS5A-D2 to CypB or CypA samples induces perturbations of chemical shifts and thus proving a direct interaction between these proteins.

Supplmental Figure 3



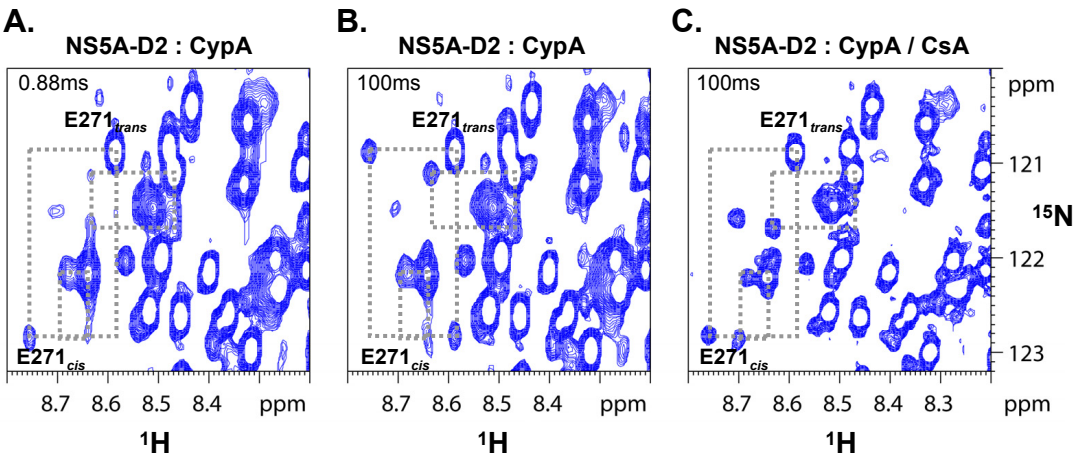
Interaction of NS5A-D2 with CypA and CypB. Each panel corresponds to the superimposition of a [^{1}H,^{15}N]-HSQC spectrum, in blue, acquired on NS5A-D2 alone and of a [^{1}H,^{15}N]-plane extracted from HNnoCO spectrum that specifically select the NS5A-D2 sub-spectrum in a NS5A-D2/Cyp (1:1) sample. Addition of Cyps causes intensive line broadening of resonances corresponding to NS5A-D2 populations where proline residues are in *cis* conformation. Furthermore several NS5A-D2 residues, in the trans populations, are differentially affected following addition of CypA or CypB.

Supplemental Figure 4



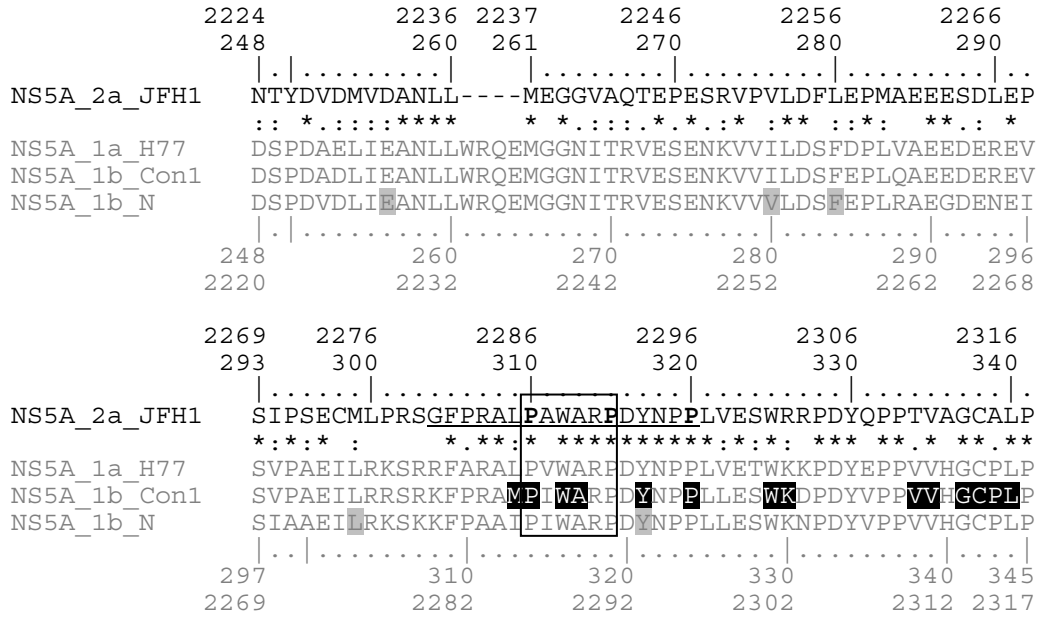
***Cis/trans* isomerization of NS5A-D2 X-Pro peptide bonds catalyzed by CypA and CypB.** $^{1}H,^{15}N$ heteronuclear exchange spectra recorded with mixing times of 0.88ms, in blue, and 100ms, in red, on [^{15}N]-NS5A-D2 samples (220µM) with catalytic amount of either CypA (**A.**) or CypB (**B.**) (23µM). The NMR resonances (*trans*, *cis* and the two exchange peaks) of NS5A-D2 residues for which the PPIase activity of a cyclophilin can be evidenced are connected by gray boxes. Exchange spectra were acquired on a 800MHz.

Supplemental Figure 5



Inhibition of PPIase activity of CypA on NS5A-D2. $^{1}H,^{15}N$ heteronuclear exchange spectra were acquired with mixing times of 0.88ms (**A.**) and 100ms (**B.**, **C.**) on a NS5A-D2:CypA (220µM:23µM) sample without (**B.**) or with an excess of CsA (**C.**). The NS5A-D2 exchange peaks due to CypA catalyzed isomerizations are no more detectable in presence of CsA, an inhibitor of cyclophilins PPIase activity.

Supplemental Figure 6



Sequences alignment of NS5A-D2 from different HCV strains. NS5A-D2 sequence of JFH1 strain (AB047639, genotype 2a) is shown in black, while the corresponding sequences of H77 (AF009606, genotype 1a), Con1 (AJ238799, genotype 1b) and N (AF139594, genotype 1b) strains are shown in grey. Sequences are numbered with respect to NS5A and the HCV polyproteins. The hyphens indicate the aa deletions comparatively to the sequence alignment. Identical, strongly conservative, and weakly conservative amino acid are indicated by asterisks, colons, and dots, respectively, accordingly to CLUSTALW convention. Prolines residues that are fully conserved in any genotypes are in bold in JFH1 sequence. The open box indicates a preferential CypA and CypB interacting region. The larger motif recognized by CypA is underlined (straight thin line).

NS5A-D2 study performed by Liang *et al.* in 2006 (37) was on genotype 1a (H77) (58.1% identity along the complete sequence and only 48.2% for domain 2). Residues reported by Tellinghuisen *et al.* in 2008 (29) to be essential for HCV replication in Con1 replicon are highlighted in black. Residues highlighted in grey correspond to those reported by Fernandez *et al.* (26) to be associated to HCV resistance to CsA in a HCV replicon of genotype 1b (AF139594). Note that the numbering of these residues in AF139594 strain is different because of a four aa insertion in the NS5A sequence.