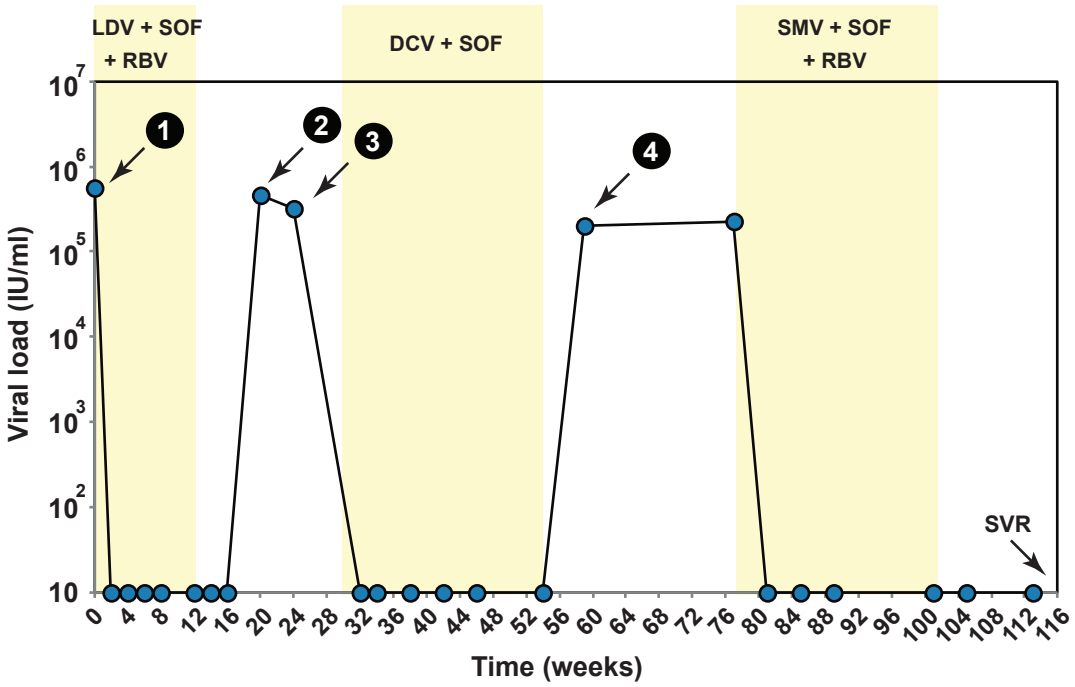
Figure S9


LDV + SOF
+ RBV
DCV + SOF
SMV + SOF
+ RBV
Viral load (IU/ml)
Time (weeks)
SVR
1
2
3
4

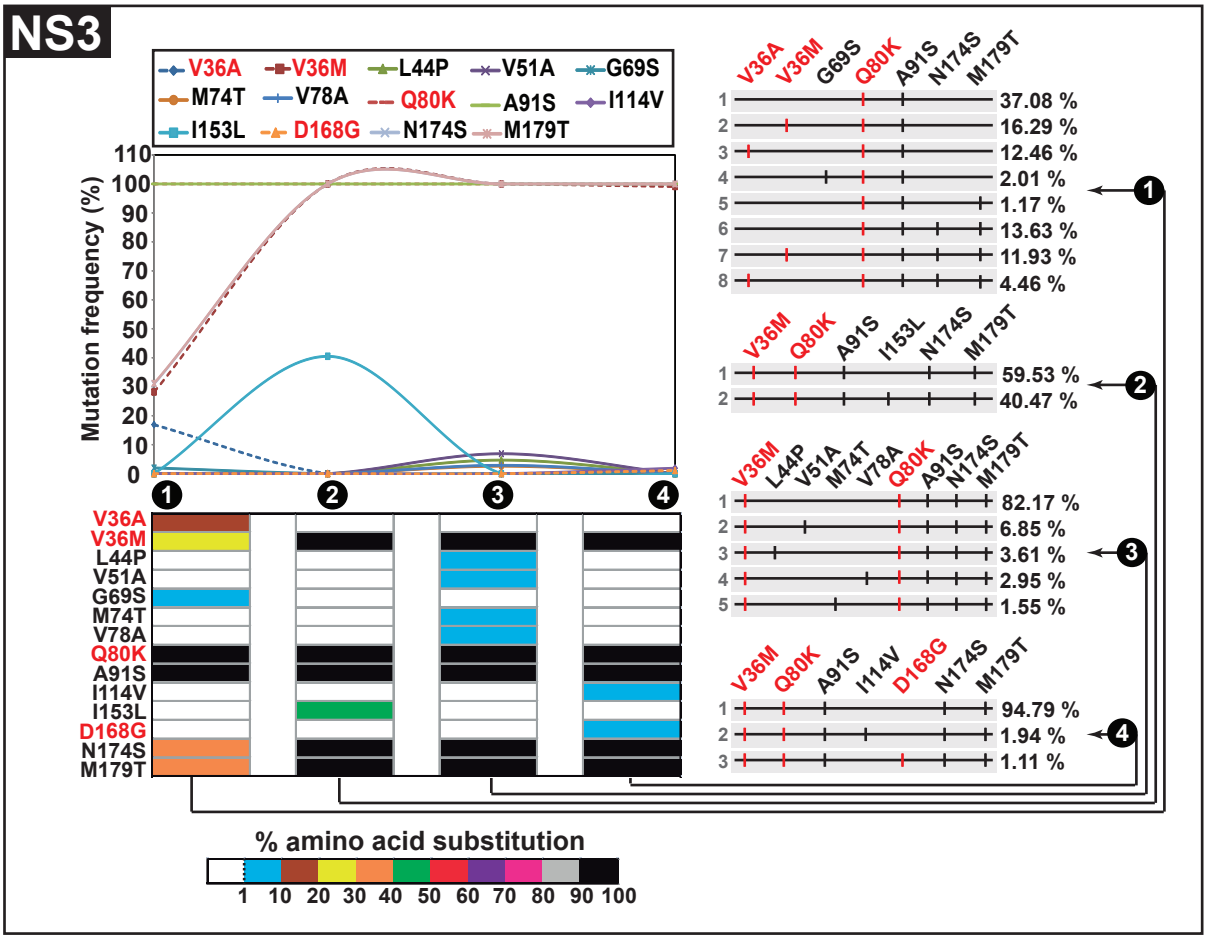
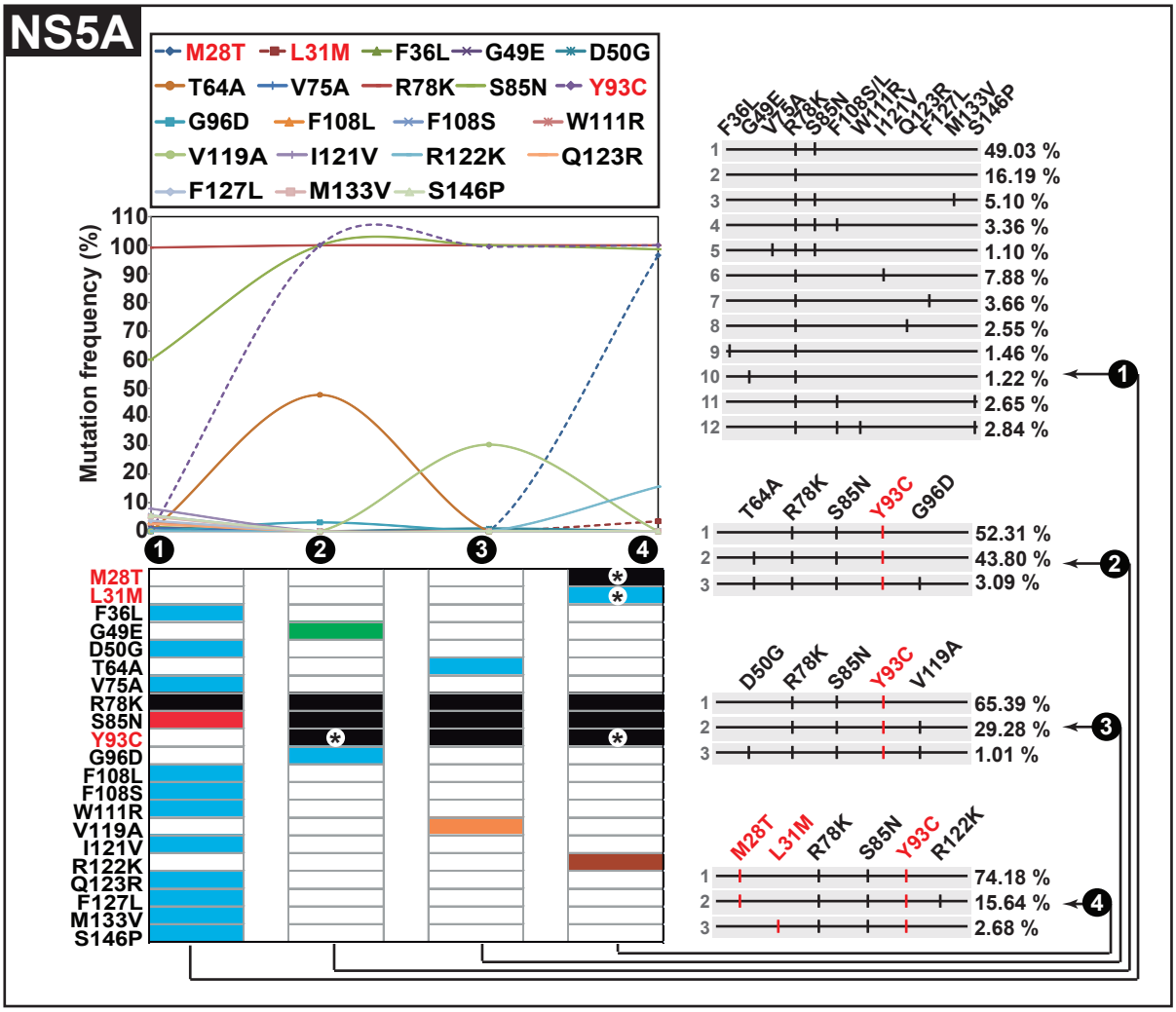

NS3
V36A V36M L44P V51A G69S
M74T V78A Q80K A91S I114V
I153L D168G N174S M179T
Mutation frequency (%)
V36A V36M G69S Q80K A91S N174S M179T
1 37.08 %
2 16.29 %
3 12.46 %
4 2.01 %
5 1.17 %
6 13.63 %
7 11.93 %
8 4.46 %
V36M Q80K A91S I153L N174S M179T
1 59.53 %
2 40.47 %
V36M L44P V51A M74T V78A Q80K A91S N174S M179T
1 82.17 %
2 6.85 %
3 3.61 %
4 2.95 %
5 1.55 %
V36M Q80K A91S I114V D168G N174S M179T
1 94.79 %
2 1.94 %
3 1.11 %
% amino acid substitution
1 10 20 30 40 50 60 70 80 90 100

Figure S9 *(continued)*



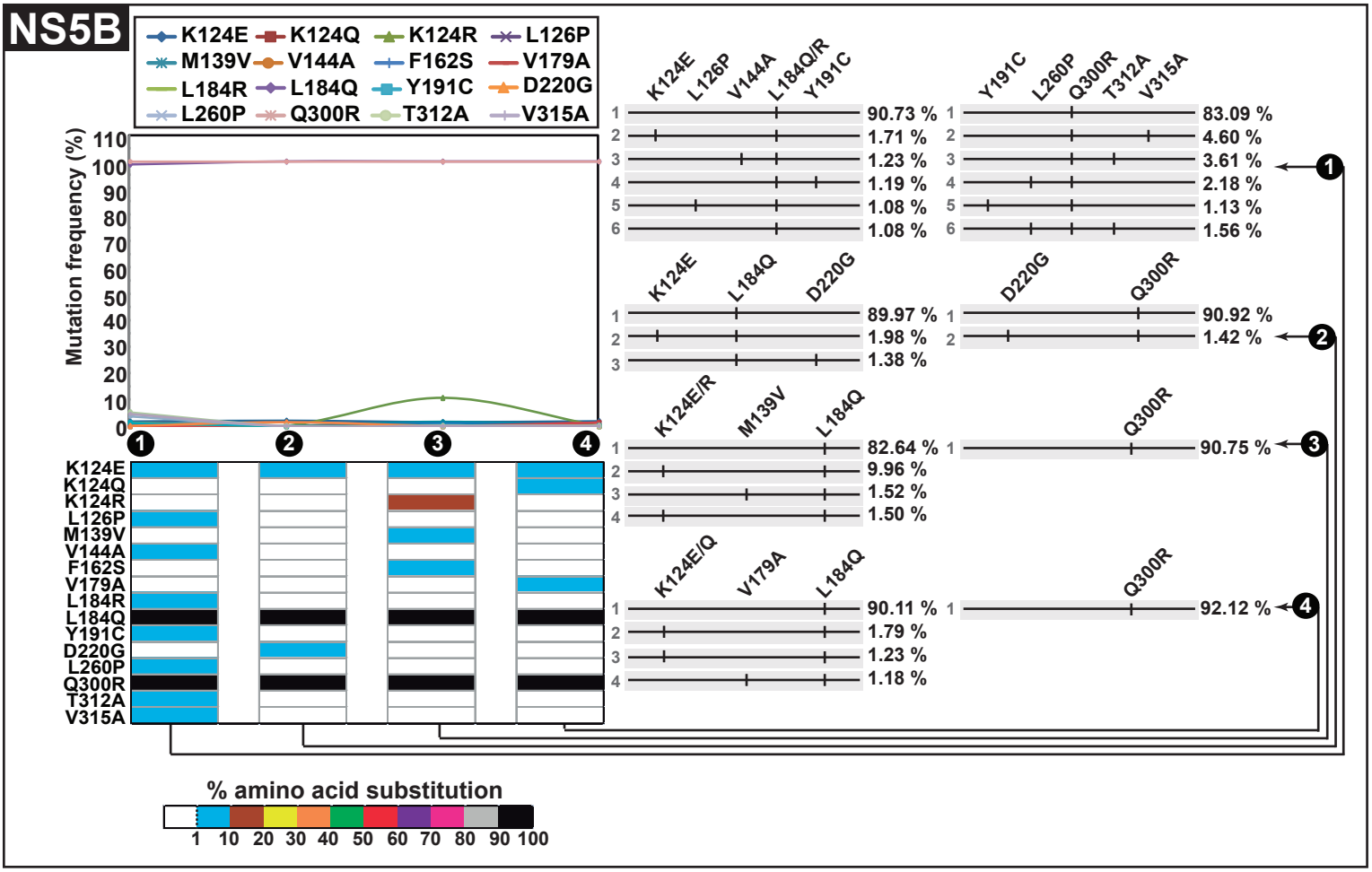


Figure S9. Ultra-deep sequencing of viral samples from an G1a HCV-infected patient subjected to sequential DAA treatments. First panel: Representation of the viral load [international units (IU)/ml] as a function of time (in weeks). A yellow background indicates period in which antiviral treatment was applied (LDV: ledipasvir; SOF: sofosbuvir; RBV: ribavirin; DCV: daclatasvir; SMV: simeprevir). Viral relapse ocurred after the first two treatments, and SVR (sustained virological response) was achieved after the third treatment. Viral RNAs from four sequential samples (termed 1 to 4, indicated with arrows) were used to amplify NS3- (PCR 1.1 in Figure 2), NS5A- (PCR 2.1 in Figure 2), and NS5B-coding regions (PCRs 3.1 and 3.2 in Figure 2) using MiSeq platforms. Subsequent panels: For each coding region (NS3, NS5A, NS5B) variations in mutation frequencies (as percentage of individual mutations) are represented as mutational waves (mutations that increase or decrease in frequency, relative to the previous or subsequent sample analyzed), and in a heat map of frequencies (color boxed below the graphics). Substitutions written in red confer resistance to the DAAs included in the treatment combinations, or are described in the literature (European Association for the Study of the Liver. Electronic address, 2017; Lontok et al., 2015; Sarrazin, 2016). Asteriscs in the heat map of NS5A-coding region in samples at viral relapse 2 and 4 highlight RAS that confer resistance to ledipasvir and daclatasvir, respectively. Haplotypes and their percentages (proportion of reads that include the indicated substitutions) are depicted on the right of each panel.