Supplementary Table 1. mAbs analyzed

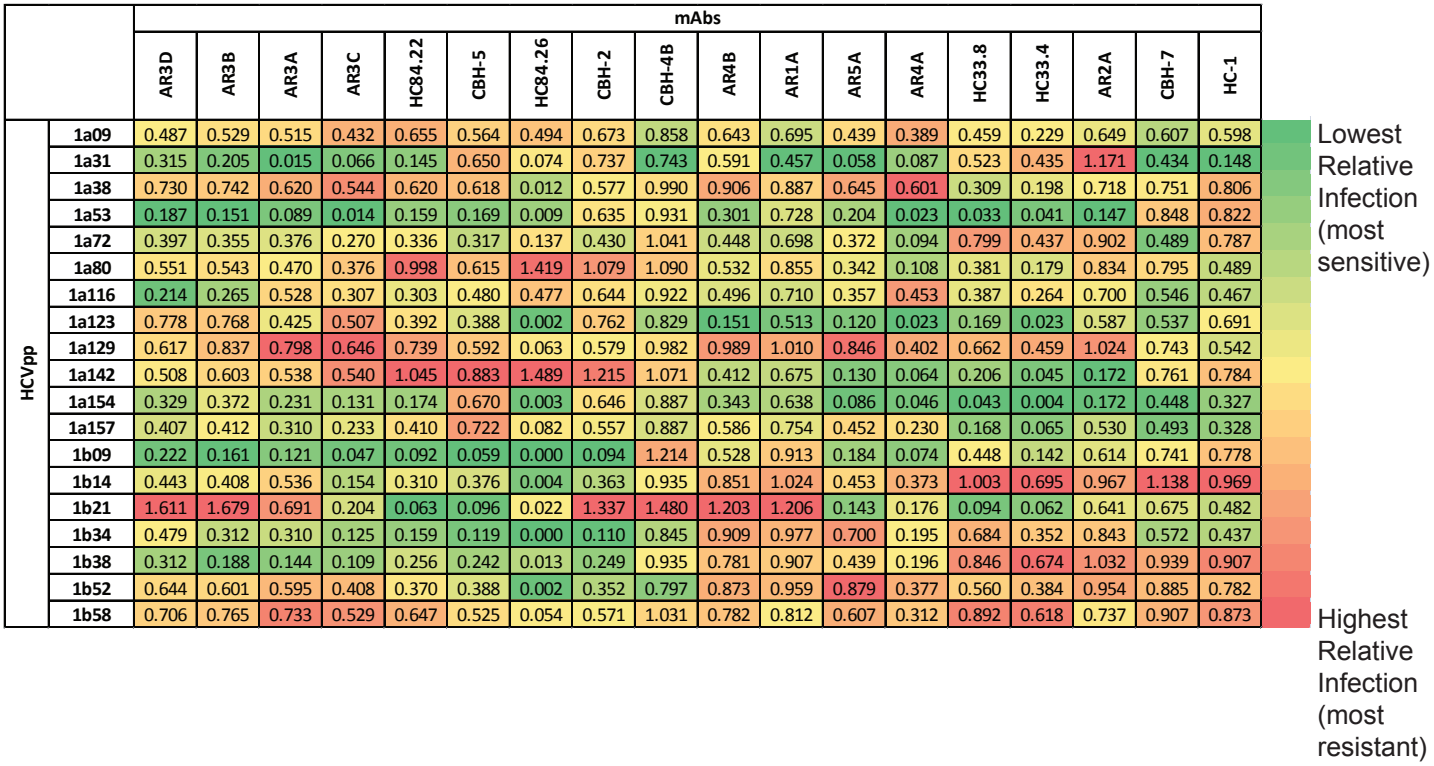
mAb	Antigenic Domains	Critical Binding Residues[1] by Alanine Scanning[2]	Citations
CBH-4B	Domain A	NA	(Hadlock, Journal of Virology, 2000), (Keck, Journal of Virology, 2004)
HC-1	Domain B	529, 530, 535, 536, 537, 540	(Keck, Journal of Virology, 2008)
CBH-2	Domain B	425, 426, 431, 434, 436, 523, 529, 530, 535, 536	(Hadlock, Journal of Virology, 2000), (Keck, Journal of Virology, 2008)
CBH-5	Domain B	412, 416, 417, 418, 420, 421, 422, 423, 483, 484, 485, 488, 523, 525, 527, 530, 533, 535, 538, 540, 550	(Hadlock, Journal of Virology, 2000), (Owsianka, J. Gen. Virol., 2008)
CBH-7	Domain C	538, 540, 547, 549	(Hadlock, Journal of Virology, 2000), (Owsianka, J. Gen. Virol., 2008)
HC84.22	Domain D	420, 426, 428, 429, 437, 441, 442, 443, 530, 616	(Keck, PLOSPathogens, 2012)
HC84.26	Domain D	429, 441, 442, 446, 616	(Keck, PLOSPathogens, 2012)
HC33.4	Epitope "1"	413, 418, 420	(Keck, Journal of Virology, 2013)
HC33.8	Epitope "1"	413, 418, 420	(Keck, Journal of Virology, 2013)
AR1A	Antigenic Region 1	416, 417, 484, 485, 538, 540, 546, 549	(Law, Nature Medicine, 2008)
AR2A	Antigenic Region 2	540	(Law, Nature Medicine, 2008)
AR3A	Antigenic Region 3/Domain B	424, 523, 525, 530, 535, 538, 540	(Law, Nature Medicine, 2008)
AR3B	Antigenic Region 3/Domain B	412, 416, 418, 423, 424, 523, 525, 530, 535, 540	(Law, Nature Medicine, 2008)
AR3C	Antigenic Region 3/Domain B	424, 488, 523, 525, 530, 535, 538, 540	(Law, Nature Medicine, 2008)
AR3D	Antigenic Region 3/Domain B	412, 424, 523, 530, 535	(Law, Nature Medicine, 2008)
AR4A	Antigenic Region 4	201, 204, 205, 206, 487, 657, 658, 692, 698	(Giang, PNAS, 2012)
AR4B	Antigenic Region 4	NA	(Giang, PNAS, 2012)
AR5A	Antigenic Region 5	201, 204, 205, 206, 639, 657, 658, 665, 692	(Giang, PNAS, 2012)

[1]Numbering based on polyprotein position

[2]Mutation of critical binding residues reported for HC84.22, HC84.26, HC33.4, HC33.8 to alanine reduce mAb binding by at least 60%. Mutation of critical binding residues reported for the remaining mAbs reduce binding by at least 50%.

NA: Not Available

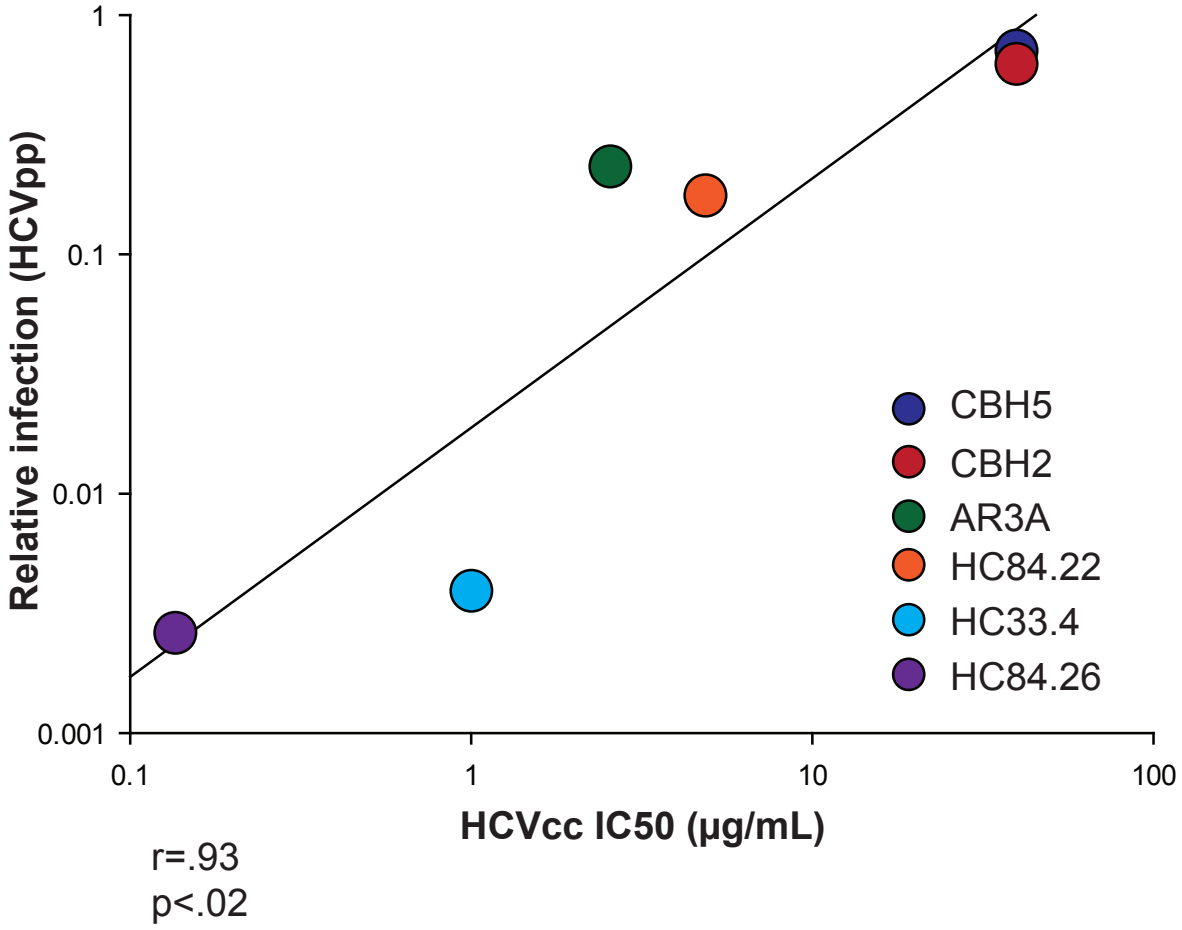
Supplementary Figure 1



		mAbs																	
		AR3D	AR3B	AR3A	AR3C	HC84.22	CBH-5	HC84.26	CBH-2	CBH-4B	AR4B	AR1A	AR5A	AR4A	HC33.8	HC33.4	AR2A	CBH-7	HC-1
HCVpp	1a09	0.487	0.529	0.515	0.432	0.655	0.564	0.494	0.673	0.858	0.643	0.695	0.439	0.389	0.459	0.229	0.649	0.607	0.598
	1a31	0.315	0.205	0.015	0.066	0.145	0.650	0.074	0.737	0.743	0.591	0.457	0.058	0.087	0.523	0.435	1.171	0.434	0.148
	1a38	0.730	0.742	0.620	0.544	0.620	0.618	0.012	0.577	0.990	0.906	0.887	0.645	0.601	0.309	0.198	0.718	0.751	0.806
	1a53	0.187	0.151	0.089	0.014	0.159	0.169	0.009	0.635	0.931	0.301	0.728	0.204	0.023	0.033	0.041	0.147	0.848	0.822
	1a72	0.397	0.355	0.376	0.270	0.336	0.317	0.137	0.430	1.041	0.448	0.698	0.372	0.094	0.799	0.437	0.902	0.489	0.787
	1a80	0.551	0.543	0.470	0.376	0.998	0.615	1.419	1.079	1.090	0.532	0.855	0.342	0.108	0.381	0.179	0.834	0.795	0.489
	1a116	0.214	0.265	0.528	0.307	0.303	0.480	0.477	0.644	0.922	0.496	0.710	0.357	0.453	0.387	0.264	0.700	0.546	0.467
	1a123	0.778	0.768	0.425	0.507	0.392	0.388	0.002	0.762	0.829	0.151	0.513	0.120	0.023	0.169	0.023	0.587	0.537	0.691
	1a129	0.617	0.837	0.798	0.646	0.739	0.592	0.063	0.579	0.982	0.989	1.010	0.846	0.402	0.662	0.459	1.024	0.743	0.542
	1a142	0.508	0.603	0.538	0.540	1.045	0.883	1.489	1.215	1.071	0.412	0.675	0.130	0.064	0.206	0.045	0.172	0.761	0.784
	1a154	0.329	0.372	0.231	0.131	0.174	0.670	0.003	0.646	0.887	0.343	0.638	0.086	0.046	0.043	0.004	0.172	0.448	0.327
	1a157	0.407	0.412	0.310	0.233	0.410	0.722	0.082	0.557	0.887	0.586	0.754	0.452	0.230	0.168	0.065	0.530	0.493	0.328
	1b09	0.222	0.161	0.121	0.047	0.092	0.059	0.000	0.094	1.214	0.528	0.913	0.184	0.074	0.448	0.142	0.614	0.741	0.778
	1b14	0.443	0.408	0.536	0.154	0.310	0.376	0.004	0.363	0.935	0.851	1.024	0.453	0.373	1.003	0.695	0.967	1.138	0.969
	1b21	1.611	1.679	0.691	0.204	0.063	0.096	0.022	1.337	1.480	1.203	1.206	0.143	0.176	0.094	0.062	0.641	0.675	0.482
	1b34	0.479	0.312	0.310	0.125	0.159	0.119	0.000	0.110	0.845	0.909	0.977	0.700	0.195	0.684	0.352	0.843	0.572	0.437
	1b38	0.312	0.188	0.144	0.109	0.256	0.242	0.013	0.249	0.935	0.781	0.907	0.439	0.196	0.846	0.674	1.032	0.939	0.907
	1b52	0.644	0.601	0.595	0.408	0.370	0.388	0.002	0.352	0.797	0.873	0.959	0.879	0.377	0.560	0.384	0.954	0.885	0.782
	1b58	0.706	0.765	0.733	0.529	0.647	0.525	0.054	0.571	1.031	0.782	0.812	0.607	0.312	0.892	0.618	0.737	0.907	0.873

Supplementary Figure 1. Each neutralizing mAb produces a "neutralization fingerprint" across the HCVpp library. Eighteen previously-characterized HCV-specific mAbs were tested for neutralization of each of the 19 clonal genotype 1a and 1b HCVpp. Four representative mAbs are shown in Figure 1, and neutralization results for all 18 mAbs are shown here. Relative infection is calculated as infection in the presence of 10 μg/ml of neutralizing mAb relative to infection in the presence of nonspecific IgG. Each value is a mean of duplicate wells. For each mAb, relative infection values are colored on a gradient, with lower relative infection values (greatest neutralization) darker green, and higher relative infection values (less neutralization) darker red. Neutralization of pseudoparticles with MLV envelope was measured as a negative control (values not shown).

Supplementary Figure 2



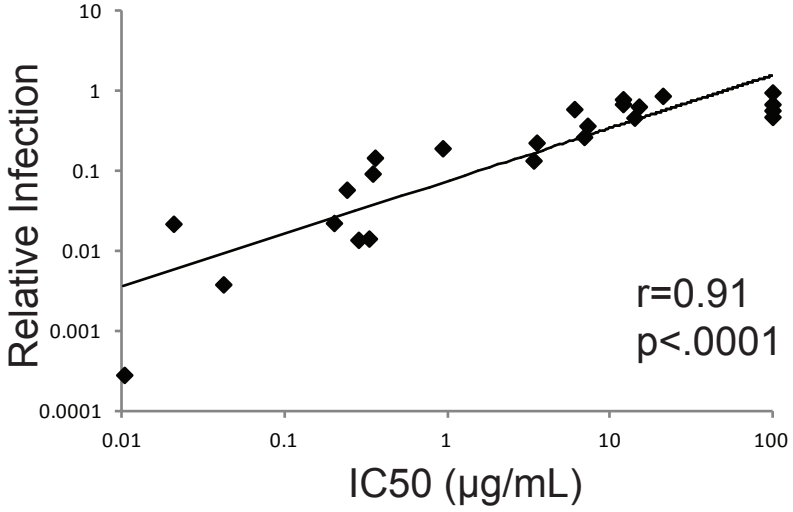
Supplementary Figure 2. Significant correlation between HCVcc IC_{50} and HCVpp relative infection. 50% inhibitory concentrations (IC_{50}'s) of the six indicated mAbs against full-length replication competent HCV (HCVcc) bearing H77 E1E2 were compared by Spearman correlation to the relative infection of HCVpp with H77 E1E2, measured using the same antibodies. Two mAbs that did not achieve 50% neutralization of HCVcc at the highest mAb concentration tested, 20 µg/mL, were assigned an IC_{50} of 40 µg/mL.

Supplementary Figure 3

a

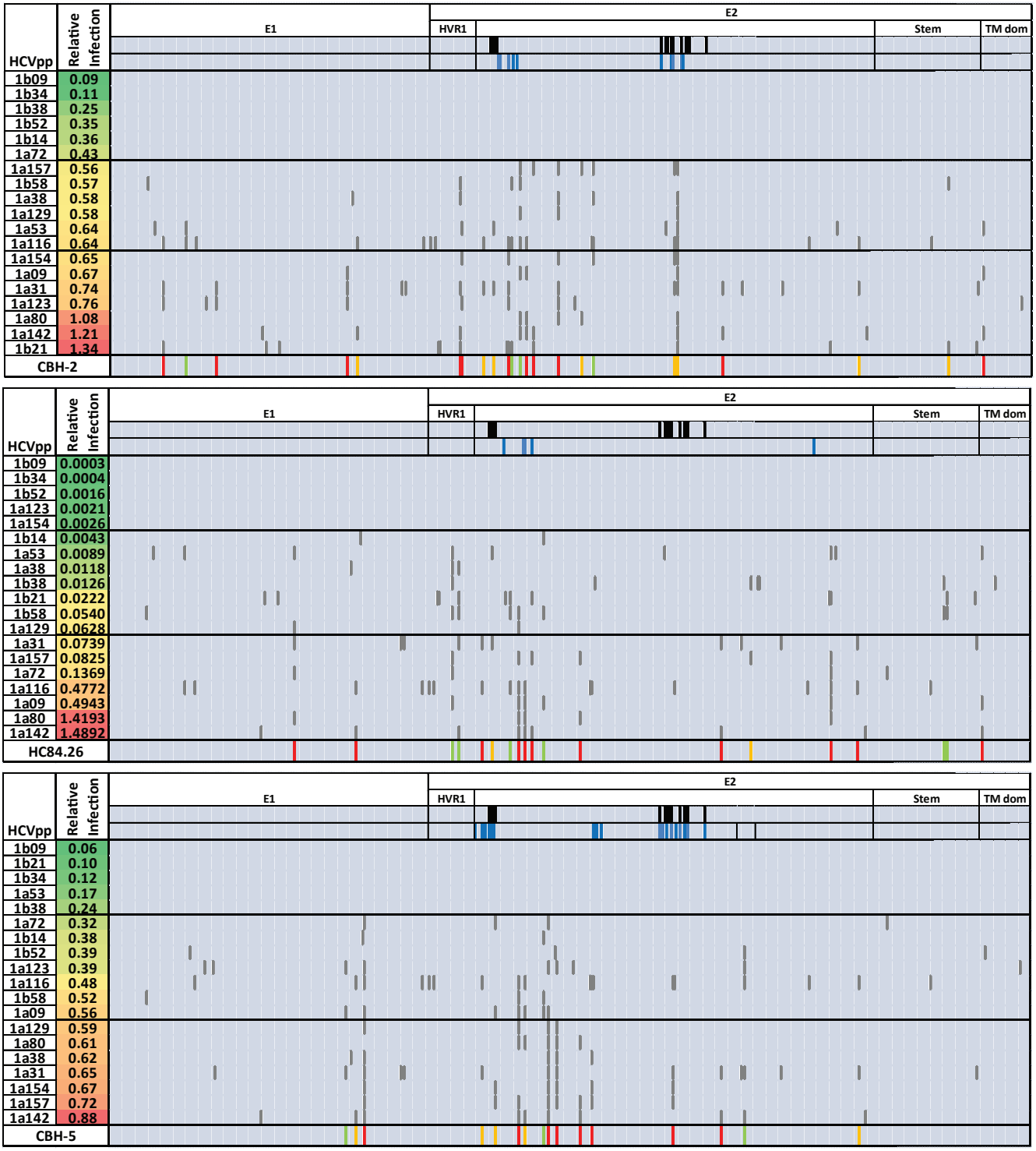
	IC50	Relative Infection
CBH-5		
pp1a116	>50	0.4801
pp1b09	0.24	0.0591
HC84.26		
pp1a72	3.38	0.1369
pp1b21	0.02	0.0222
pp1b09	0.01	0.0003
CBH-2		
pp1a129	>50	0.5788
pp1b09	0.35	0.0940
HC-1		
pp1b14	>50	0.9688
pp1a123	>50	0.6909
pp1a31	0.36	0.1485
HC33.4		
pp1b14	12.02	0.6946
pp1a09	3.53	0.2287
pp1a154	0.04	0.0039
AR3A		
pp1a129	12.02	0.7978
pp1a80	14.13	0.4698
pp1a31	0.33	0.0145
AR3C		
pp1a129	15.05	0.6461
pp1a72	6.93	0.2697
pp1a53	0.28	0.0140
AR4A		
pp1a38	6.03	0.6010
pp1b34	0.93	0.1947
pp1a53	0.20	0.0227
AR5A		
pp1b52	21.12	0.8793
pp1a72	7.26	0.3718

b



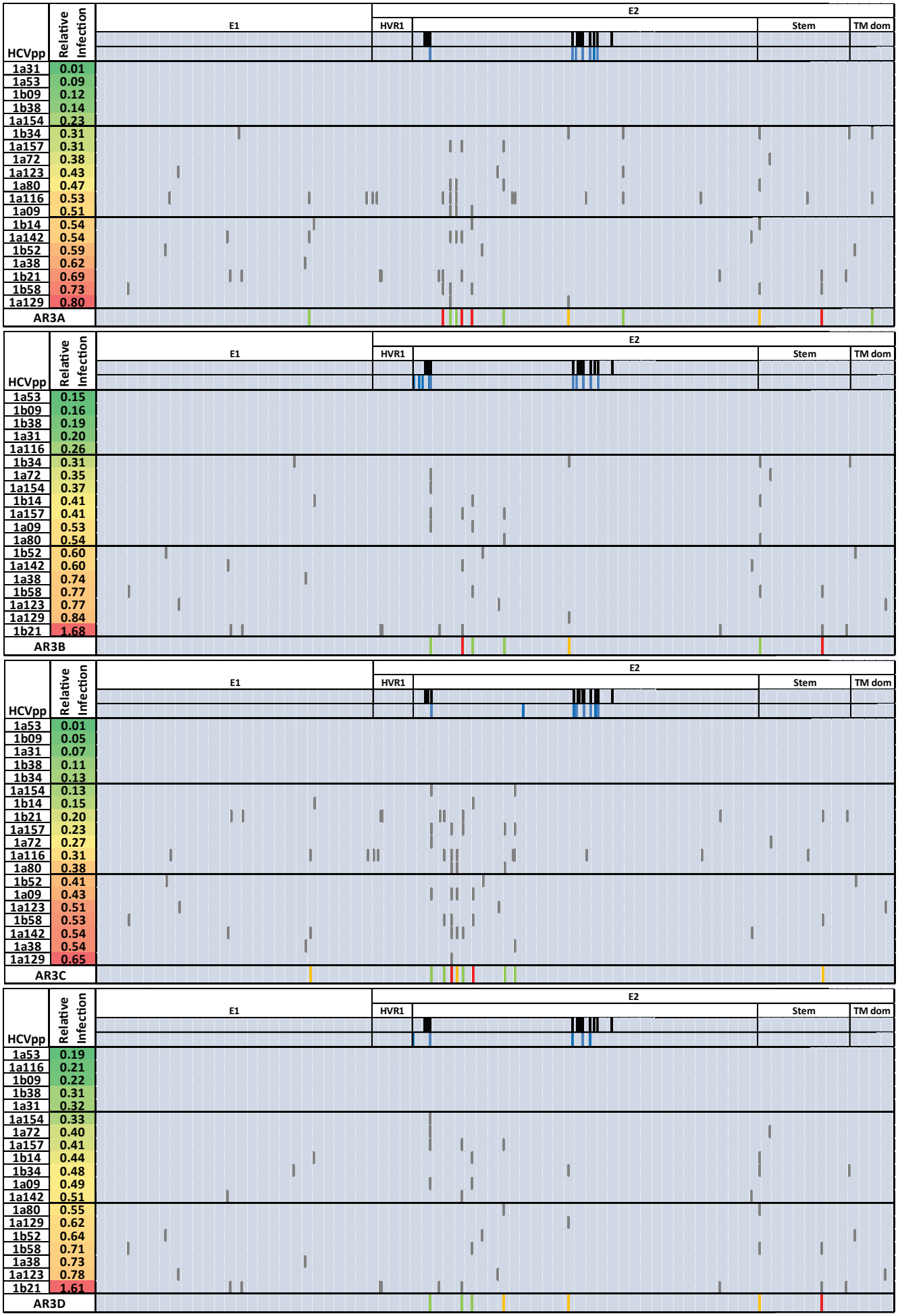
Supplementary Figure 3. Significant correlation between HCVpp IC_{50} and HCVpp relative infection. **(a)** All combinations for which both relative infection at 10 µg/mL of mAb and IC_{50} were measured in independent experiments. **(b)** Correlation between relative infection value and IC_{50} for the same HCVpp/mAb combination. Each point represents one mAb/HCVpp combination. MAbs that did not achieve 50% neutralization at the highest mAb concentration tested, 50 µg/mL, were assigned an IC_{50} of 100 µg/mL. Spearman correlation (r)=0.91 with p<0.0001.

Supplementary Figure 4

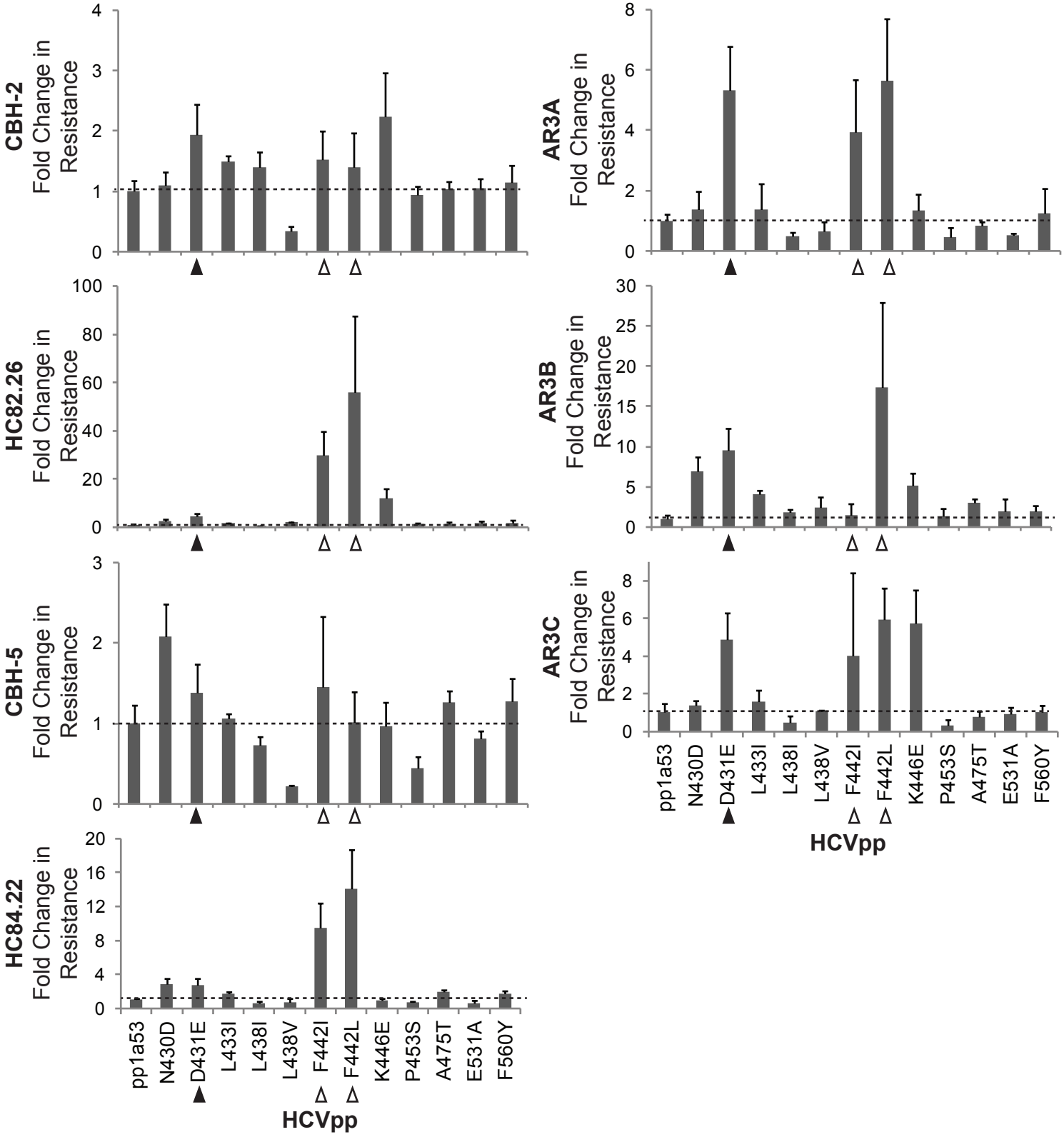


Supplementary Figure 4. Sequence analysis reveals resistance-associated polymorphisms. Sequence analysis for NC1 mAbs not shown in Figure 3. Clones are grouped into the 5 most sensitive, 7 with intermediate resistance, and 7 with greatest resistance, separated by horizontal black lines. Gray vertical bars indicate positions with a substitution in any resistant E1E2 clone but in none of the five most sensitive E1E2 clones. Black vertical bars indicate CD81 binding sites in E2. Blue vertical bars indicate critical binding residues for the indicated mAb, determined by alanine scanning. Sites with substitutions in at least two resistant clones but in no sensitive clones are included in the summary panel in the bottom row. Sites marked with vertical red bars are predominantly polymorphic in the seven most resistant clones. Orange vertical bars indicate sites that are polymorphic in an equal number of highly resistant and intermediate resistant clones. Green vertical bars indicate sites that are predominantly polymorphic in the seven clones with intermediate resistance.

Supplementary Figure 4 (Continued)

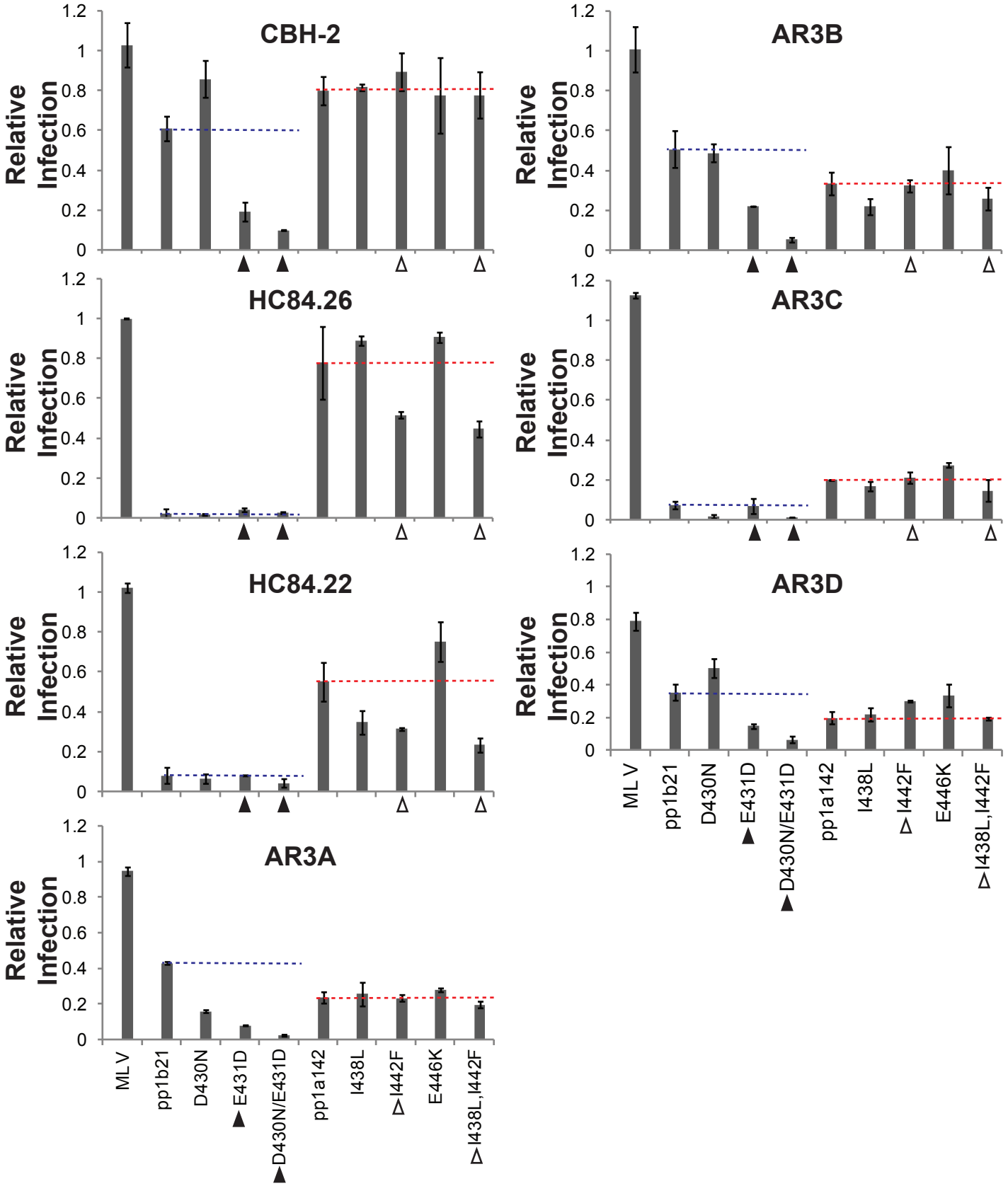


Supplementary Figure 5



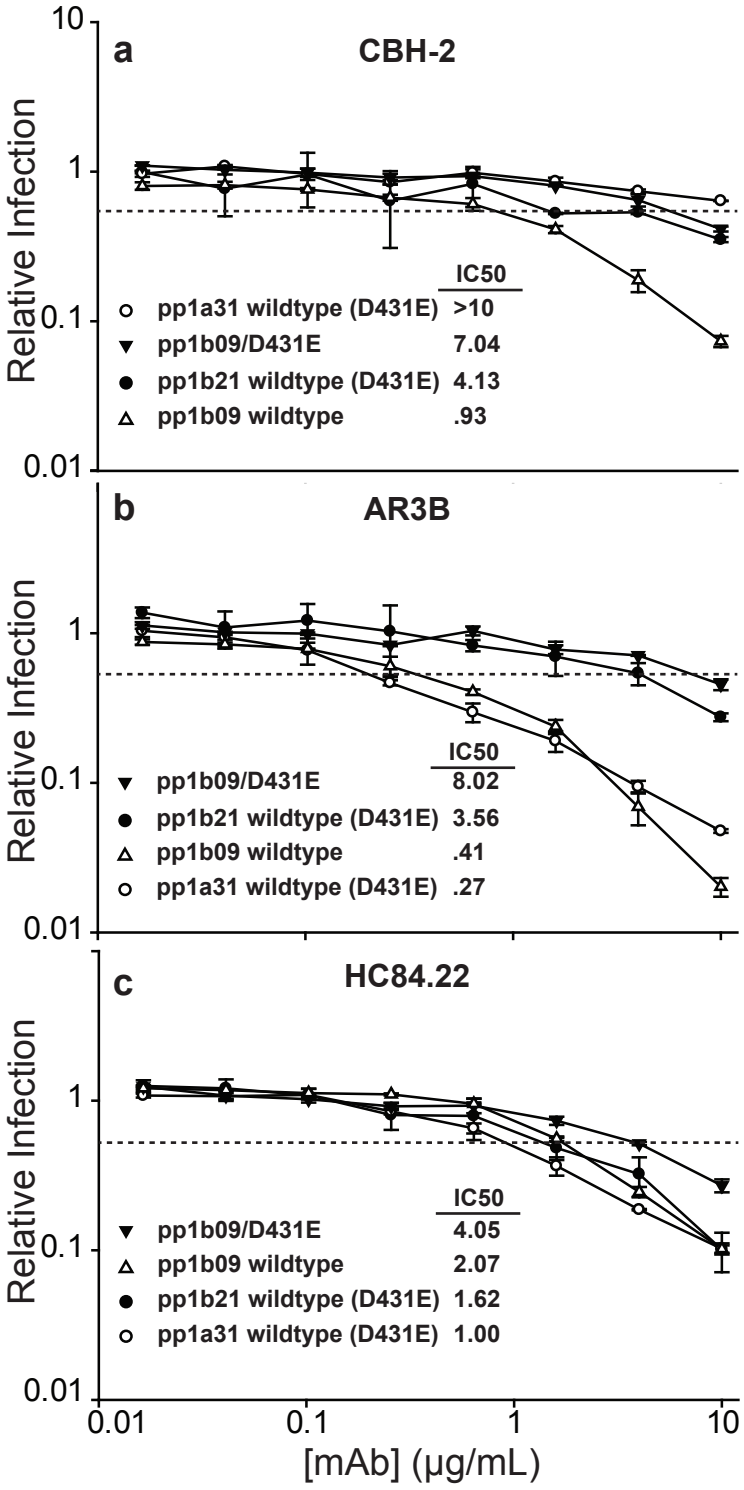
Supplementary Figure 5. Introduction of resistance-associated polymorphisms into a second neutralization sensitive E1E2 clone (1a53) confirms the resistance phenotypes of D431E and F442I. Eleven mutations of interest were introduced by site directed mutagenesis into an E1E2 clone that was sensitive to neutralization by each of the NC1 mAbs (clone 1a53). The dashed line indicates relative infection of HCVpp using wildtype 1a53 in the presence of the indicated mAb, adjusted to 1. Each bar indicates the fold change in neutralization resistance after the indicated mutation(s) were introduced into clone 1a53. Error bars indicate standard deviation between duplicate wells. Black triangles indicate HCVpp with D431E mutations. Open triangles indicate HCVpp with F442I or F442L mutations.

Supplementary Figure 6



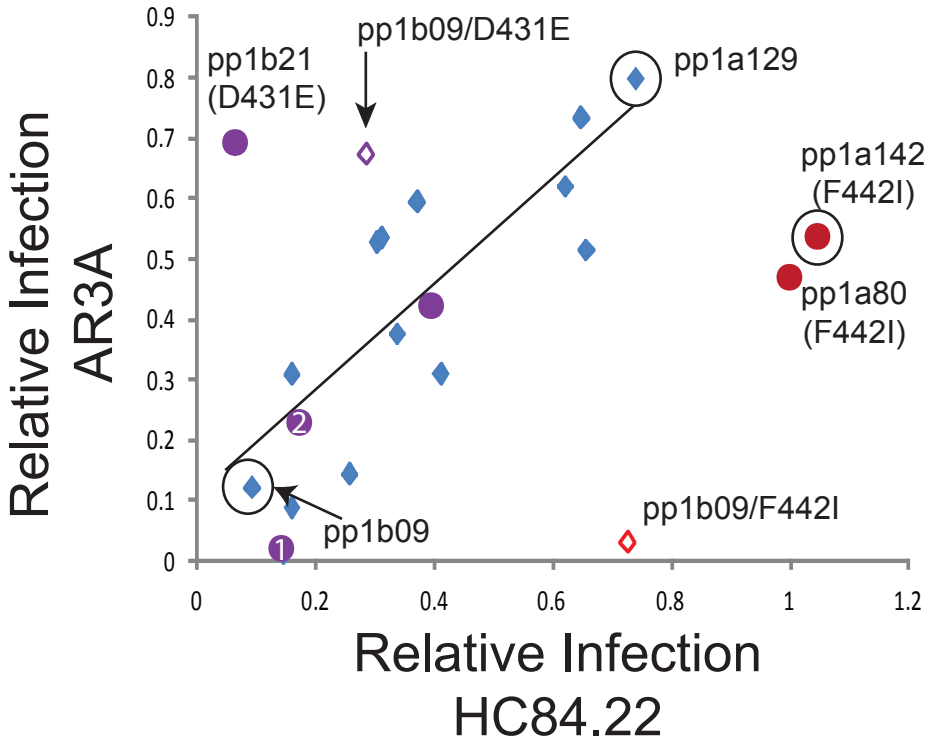
Supplementary Figure 6. Back-mutation of resistance-associated polymorphisms in E1E2 clones where they appear naturally. Each bar indicates relative infection of the indicated HCVpp in the presence of the indicated mAb. 1b21 is an E1E2 clone containing naturally occurring N430D and D431E. The dashed blue line indicates relative infection of pp1b21 prior to introduction of D430N, E431D, or both mutations. 1a142 is an E1E2 clone containing naturally occurring L438I, F442I, and K446E. The dashed red line indicates relative infection of pp1a142 prior to introduction of I438L, I442F, E446K, or I438L/I442F. Error bars indicate standard deviation between duplicate wells.

Supplementary Figure 7



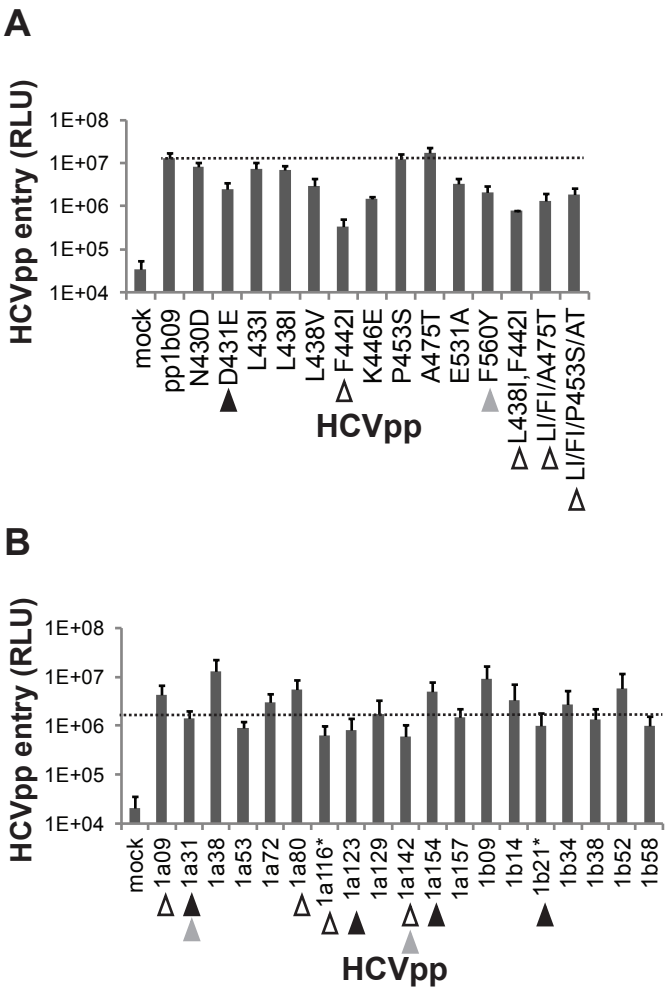
Supplementary Figure 7. Resistance conferred by D431E is E1E2 context-specific. IC50's were measured against HCVpp using two E1E2 clones with naturally-occurring D431E (1b21 and 1a31), a naturally occurring neutralization sensitive E1E2 clone (1b09), and 1b09 with D431E introduced by site-directed mutagenesis (1b09/D431E). **(a)** Mutation of D431 to E confers CBH-2 resistance to clone 1b09, and all E1E2 variants with D431E were CBH-2 resistant. **(b)** Mutation of D431 to E confers AR3B resistance to clone 1b09, and the 1b21 clone with naturally occurring D431E is resistant to AR3B as well. Clone 1a31, which also contains a naturally occurring D431E, is not resistant to AR3B. **(c)** Mutation of D431 to E also confers HC84.22 resistance to clone 1b09. However, clones 1a31 and 1b21, which also carry D431E, are not resistant to HC84.22. Dashed lines indicate 50% neutralization. Error bars indicate standard deviation between duplicate wells.

Supplementary Figure 8



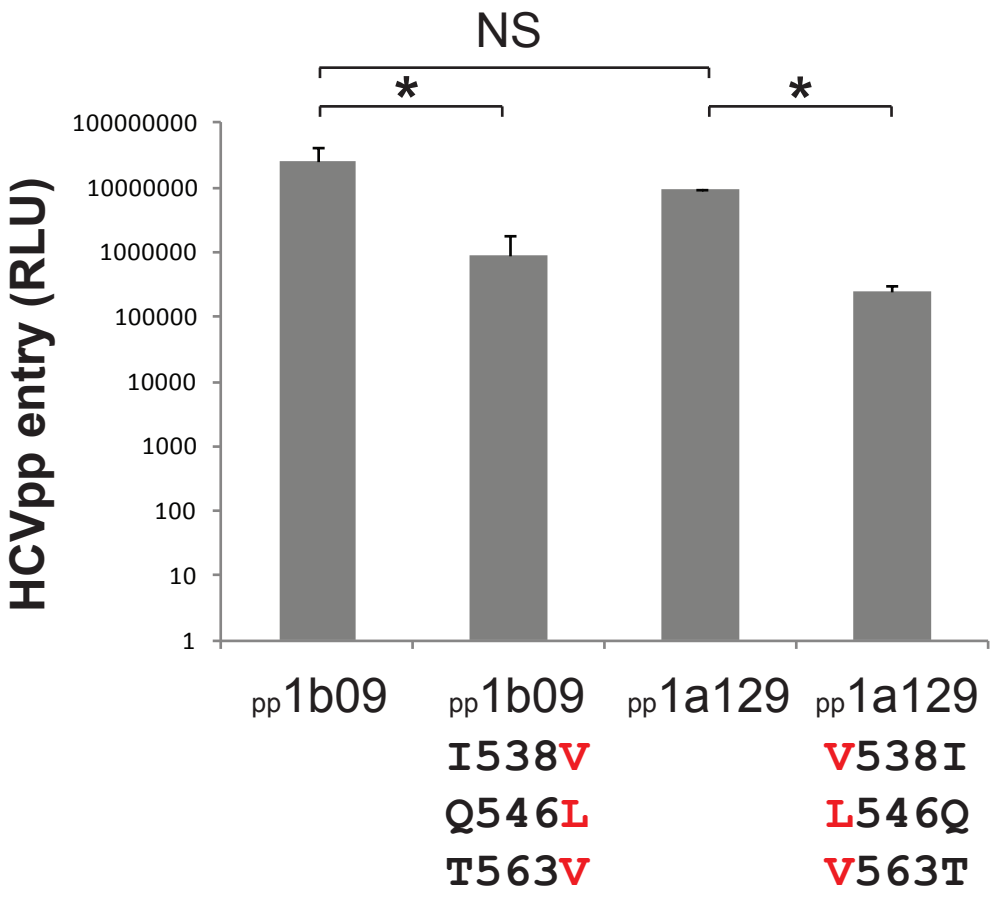
Supplementary Figure 8. D431E and F442I polymorphisms are present in outliers on the neutralization correlation plot between mAbs HC84.22 and AR3A. Each diamond or colored circle indicates the neutralization of a single HCVpp by mAb HC84.22 on the x-axis and mAb AR3A on the y-axis. D431E confers relatively greater resistance to AR3A, while F442I confers relatively greater resistance to HC84.22. Solid purple circles indicate clones with naturally occurring D431E polymorphisms. Solid red circles indicate clones with naturally occurring F442I polymorphisms. Open purple and red diamonds indicate clone 1b09 with D431E or F442I, respectively, introduced by site directed mutagenesis. Two clones (1a142 and 1a80) with greater resistance to HC84.22 than to AR3A carry naturally-occurring F442I polymorphisms. A clone (1b21) with greater resistance to AR3A than to HC84.22 carries a naturally-occurring D431E polymorphism. Two other clones with naturally-occurring D431E (numbered “1” and “2”) are relatively sensitive to both AR3A and HC84.22. The E1E2 clone from the panel with highest combined resistance to both AR3A and HC84.22 (1a129) does not carry either D431E or F442I. E1E2 chimeras used in Figures 5 and 6 were generated between the circled clones on this plot.

Supplementary Figure 9



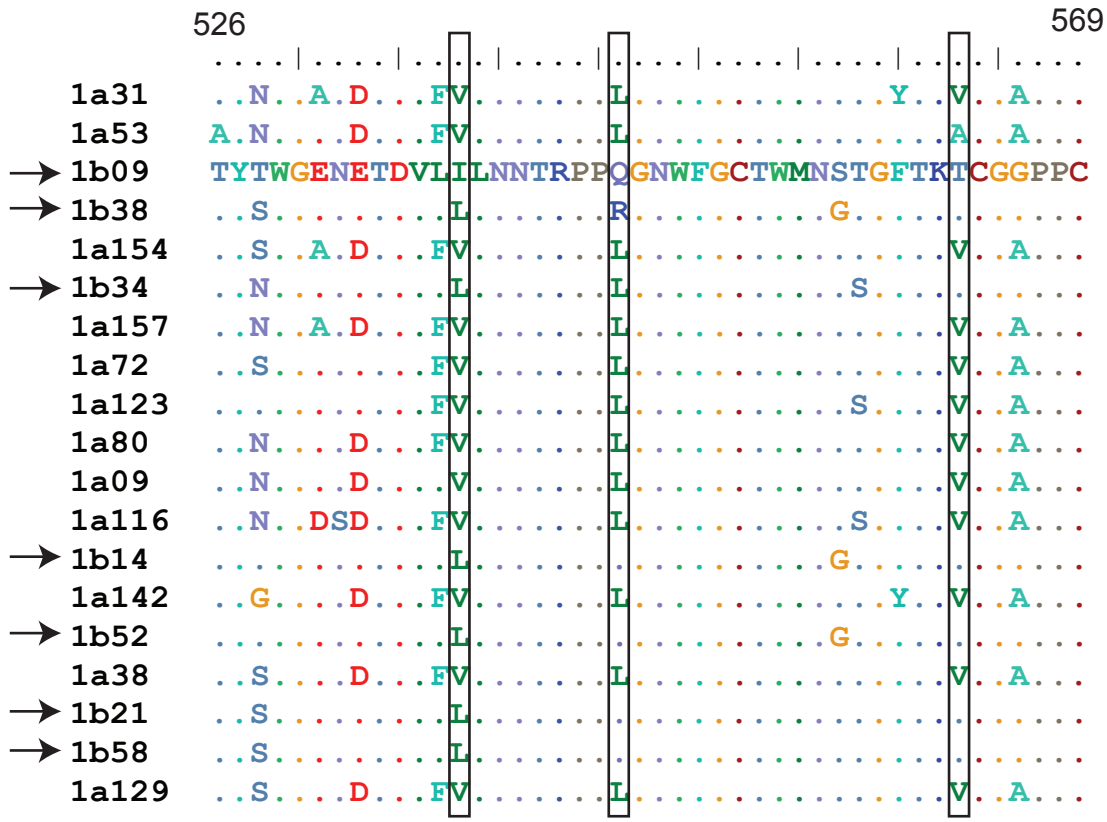
Supplementary Figure 9. Additional polymorphisms can compensate for fitness cost of resistance polymorphisms. (A) The effect of site-directed mutations on 1b09 E1E2 fitness (ability to mediate HCVpp entry). RLU are relative light units, indicating productive entry of HCVpp into target cells. Values are means of two independent experiments performed in duplicate. Error bars indicate standard deviations. The dotted line indicates entry of HCVpp with wildtype 1b09 E1E2. **(B)** HCVpp entry mediated by naturally-occurring E1E2 clones in the HCVpp panel. Mock pseudoparticles with no E1E2 were used as a negative control. Solid triangles indicate HCVpp with naturally-occurring D431E polymorphisms. Open triangles indicate HCVpp with naturally-occurring F442I or F442L. Gray triangles indicated HCVpp with naturally-occurring F560Y. Values are the means of four to eleven independent experiments performed in duplicate. Error bars indicated standard deviations. The dotted line indicates median entry of all 19 HCVpp. HCVpp indicated with asterisks were never frozen prior to testing. The remaining HCVpp were freeze-thawed once prior to testing.

Supplementary Figure 10



Supplementary Figure 10. Fitness cost of I538V/Q546L/T563V polymorphisms is compensated in a resistant E1E2 clone where it arises naturally. Each bar represents entry by HCVpp with the indicated E1E2s. Values are means of two to six independent experiments performed in duplicate. RLU is relative light units. Error bars are standard deviations. An asterisk indicates $p<.05$ by T test. NS indicates p value not significant. Introduction of I538V/Q546L/T563V reduces fitness of clone 1b09, but clone 1a129, with naturally-occurring I538V/Q546L/T563V, does not exhibit reduced fitness, and reversion of these polymorphisms in clone 1a129 also reduces fitness. Therefore, fitness cost of these polymorphisms is E1E2 context dependent.

Supplementary Figure 11



Supplementary Figure 11. Neutralization resistance conferred by I538V/Q546L/T563V is E1E2 context dependent. Sequence alignment of amino acids 526-569 for all E1E2 clones in the HCVpp panel. Clones are listed by increasing resistance to mAb AR3A. Boxes indicate residues 538, 546, and 563. Dots indicate homology to sensitive clone 1b09 at that site. Arrows indicate genotype 1b clones. The I538V/Q546L/T563V combination of polymorphisms is common in genotype 1a isolates in the E1E2 panel and not independently associated with NC1 mAb resistance.