

1 **Supplemental figure legends**

2 **Figure S1. Peptides selected by mRNA display-HTS using FLAG M2 mAb.** The most  
3 abundant peptides selected by FLAG M2 mAb and their copy numbers are shown (among ~1  
4 million total sequences obtained after 2 rounds of mRNA display selection). Residues that are  
5 identical to the FLAG epitope DYKDDDDK are in bold.

6 **Figure S2. Acid residues are present at a high frequency adjacent to the W(L/I) motif.**

7 Peptide sequences obtained from the 4<sup>th</sup> round of selection (copy numbers > 100, ~2000 clones)  
8 were used for analysis. Sequences that contain the W(L/I) motif were selected and the amino  
9 acid residue frequencies were calculated in the flanking regions. Residue W was set as position 0  
10 and its frequency 100%. Background residue frequencies are less than 10% (not shown). Acidic  
11 residues D and E show high frequencies at the +2 position. Residue G shows high frequency at  
12 position -1, and the F residue also showed a frequency higher than the background at position +5.  
13 Both G and F are present in the original wild type sequence

14 **Figure S3. The most abundant peptides selected by mAb41 using a 15-mer library.** Copy

15 numbers are among ~1 million total sequences obtained after 2 rounds of mRNA display  
16 selection). QLRNSCA is the constant region. Dashes are artificially introduced to show  
17 sequence alignments. The W(L/I) or W(L/I)XX(L/I) motifs are in bold. Acidic residues next to  
18 the W(L/I) motif are Italicized. The upstream G residue (Italicized) was also identified.

19 **Figure S4. Binding of selected peptides to mAb41. (A)** Competition for binding to mAb41

20 between p41\_3 and wild type pB. Binding was carried out as described in **Figure. 3. (B-E)**  
21 Sensorgrams (blue) obtained by Octet RED and curve fitting (red, 1:1 model) for binding of the  
22 mAb41 Fabs to peptides **(B)** pB, **(C)** p41\_3, **(D)** p41\_4, and **(E)** p41\_5.

23 **Figure S5. Assessment of the role of the acidic residues in binding of selected peptides to**  
24 **mAb41.** (A) Biotinylated mutant peptides were synthesized. p41\_1\_EtoA carries an E to A  
25 mutation within the second WLXXL motif of p41\_1. p41\_2\_QtoD carries a Q to D mutation in  
26 the WLQEI motif of p41\_2. (B-C) Binding of the p41\_1 (B) and p41\_2 (C) mutant peptides to  
27 mAb41 was measured by ELISA in comparison with the original peptides. ELISA was  
28 performed as described in **Figure 3** except that mAb 41 was applied as primary antibody in a 2-  
29 fold dilution series.

30 **Figure S6. Neutralization of HCVcc GT1a/2a chimeric virus.** Polyclonal sera from mice  
31 immunized with peptides (A) p41\_1, (B) p41\_3, (C) p41\_4, (D) p41\_5, (E) pA, or (F) NMS  
32 were tested for HCVcc GT1a/2a neutralizing activities. For each serum sample, four dilutions of  
33 1:20, 1:40, 1:80 and 1:160 were used.

SFig1

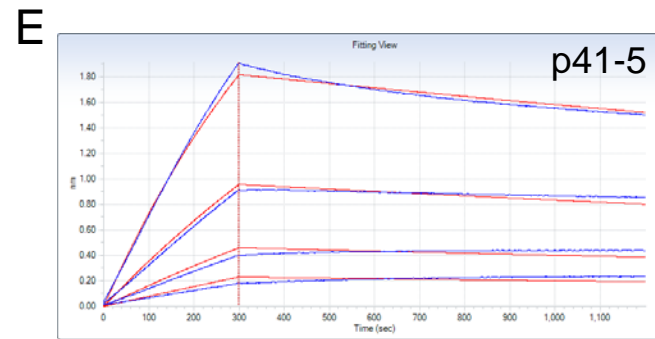
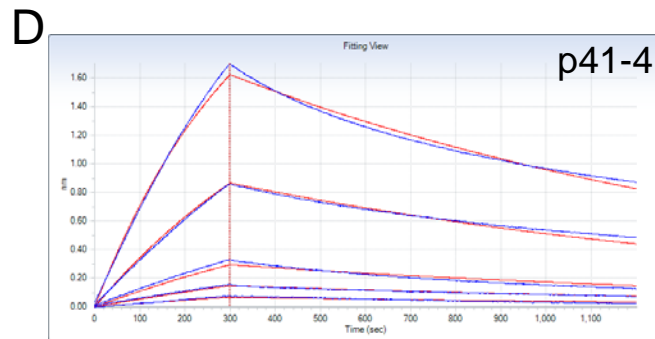
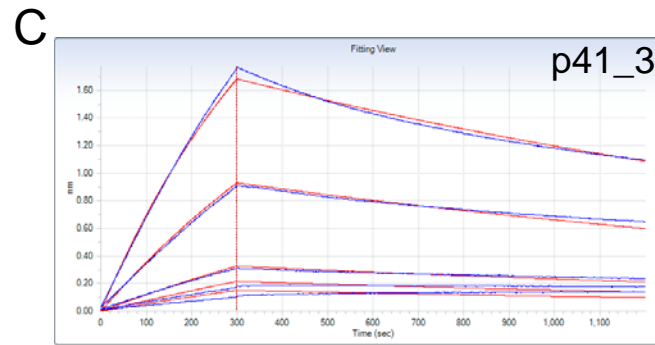
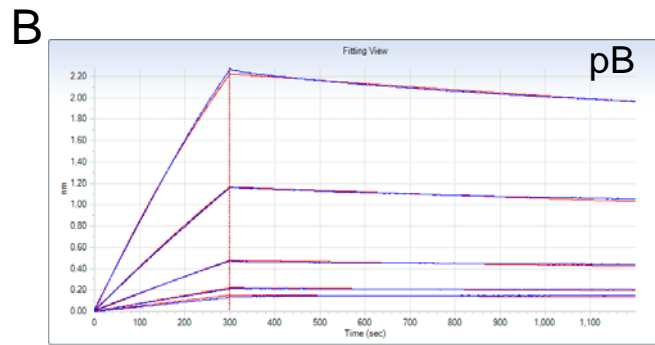
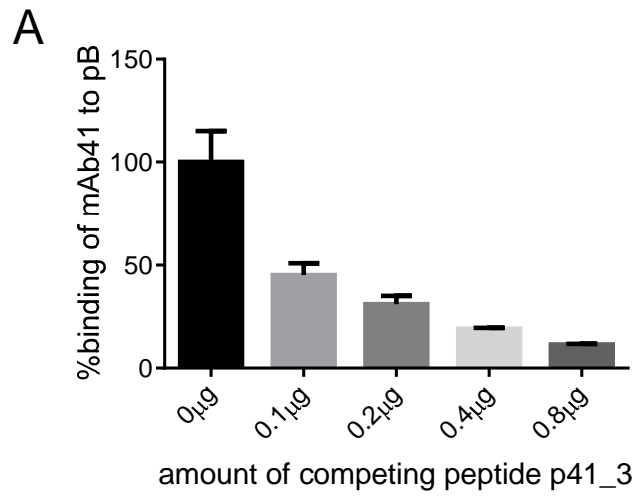
Peptides selected by anti-Flag M2	Copies
MQICND <b>DYKDDD</b> VKNSVQLRNSCA	16
MYKLMDR <b>SDYKYAD</b> THQLRNSCA	13
MCYGD <b>DYKNGD</b> LTQLRNSCA	9
MSYPCDIPT <b>F</b> DYKMT <b>D</b> QLRNSCA	9
MRTRMKNP <b>DYKNED</b> CQLRNSCA	8
...	...

SFig2

Positions Residues	X -1	W 0	[LI] +1	X +2	X +3	X +4	X +5
W		100%					
L			77.4%			60.6%	
I			22.6%			10.6%	
D				33.7%			
E				28.5%			
G	43.2%						
F							12.2%

SFig3

Peptides selected by mAb41 using a 15-mer library	Copies
M <b>W</b> IHG-----G <b>W</b> LE <b>T</b> LTTC <b>S</b> G <b>Q</b> LRNSCA	10
MLWYHKCANW---G <b>W</b> L <b>N</b> S <b>L</b> Q <b>L</b> RNSCA	8
MCDRKSGLLWNY---G <b>W</b> LE <b>Q</b> LRNSCA	6
MKES <b>W</b> ID <b>G</b> I <b>W</b> RE---G <b>W</b> LE <b>Q</b> LRNSCA	6
MIWEYNTG <b>W</b> L <b>D</b> S <b>I</b> W <b>V</b> S <b>Q</b> LRNSCA	5
M <b>W</b> L <b>C</b> W <b>N</b> Y <b>D</b> K <b>N</b> K <b>T</b> G <b>W</b> L <b>D</b> Q <b>L</b> RNSCA	5
...	...



**A**

Names	Sequences
p41_1	MMECEKGGKYVQGWLCHLFWEDCQDRGWLEQLRNSCA
P41_1_EtoA	MMECEKGGKYVQGWLCHLFWEDCQDRGWLAQLRNSCA
P41_2	MRYEPMMMNCSPAWLQEIVQWLNEDWPEQLRNSCA
P41_2_QtoD	MRYEPMMMNCSPAWLDEIVQWLNEDWPEQLRNSCA

