

1 **SUPPORTING INFORMATION for**

2 **Probing the impact of local structural dynamics of conformational epitopes on**

3 **antibody recognition**

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10 The supporting information includes data for protein purification and the complete  
11 hydrogen/deuterium-exchange mass spectrometry data in the form of butterfly plots and  
12 deuterium up-take plots for each observable peptide. A peptic digest sequence coverage map is  
13 also included. Lastly, Octet biolayer interferometry sensorgrams are provided.

14 **Supporting information contents:**

15 **Figure S1** SDS-PAGE, native PAGE and gel filtration analysis of purified FL,  $\Delta V3$ ,  $\Delta V1/V2$   
16 and Core<sub>e</sub>

17 **Figure S2** Observable peptides coverage map of unliganded full-length YU2 gp120

18 **Figure S3** Structural dynamics in gp120 monomers deleted with variable loops detected by H/D  
19 exchange shown in butterfly plots

20 **Figure S4** Structural dynamics in gp120 monomers deleted with variable loops compared to  
21 FL+sCD4 as detected by H/D exchange

22 **Figure S5** Deuterium exchange profiles for the various observable peptides of gp120s

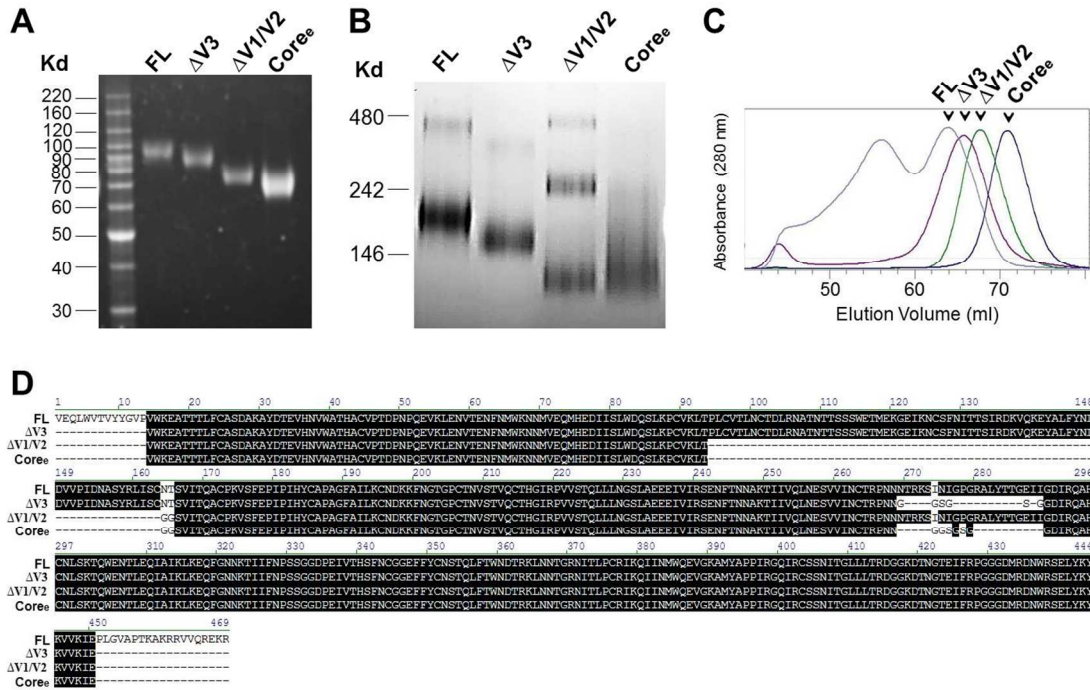
23 **Figure S6** Comparisons of H/D exchange profiles of  $\Delta V3$ ,  $\Delta V1/V2$  and Core<sub>e</sub>

24 **Figure S7** Octet biolayer interferometry binding curves for CD4i binding antibodies

25 **Figure S8** Octet biolayer interferometry binding curves for CD4-IgG2 and CD4BS binding  
26 antibodies

27

28 **Figure S1.** SDS-PAGE, native PAGE and gel filtration analysis of purified FL,  $\Delta V3$ ,  $\Delta V1/V2$   
 29 and Core<sub>e</sub>. (A) SDS-PAGE analysis of purified gp120s. (B) Blue native-PAGE analysis of  
 30 purified gp120s. (C) Gel filtration analysis of purified gp120s. (D) Sequence alignment for FL,  
 31  $\Delta V3$ ,  $\Delta V1/V2$  and Core<sub>e</sub>.



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39 **Figure S2.** Observable peptides coverage map of unliganded full-length YU2 gp120. The  
 40 primary gp120 sequence is underlined according to the observable peptide fragments used for  
 41 H/D exchange measurements. The inner domain residues are shown in pink. The outer domain  
 42 residues are shown in grey. The bridging sheets residues are shown in red. V1-V5 loops and the  
 43 CD4 binding loop are highlighted as yellow.

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  1  VEQLWVTVYY GVPVWKEATT TLFCASDAKA YDTEVHNVWA THACVPTDPN
  51  PQEVKLENT ENFNMWKNM VEQMHEDIIS LWDQSLKPCV KLTPLCVTLN
      v1                                v2
 101  CTDLRNATNT TSSSWETMEK GEIKNCSFNI TTSIRDKVQK EYALFYNLDV
 151  VPIDNASYRL ISCNTSVITQ ACPKVSFEPI PIHYCAPAGF AILKCNDKKE
 201  NGTGPCTNVS TVQCTHGIRP VVSTQLLNG SLAEEEIVIR SENFTNNAKT
      v3
 251  IIVQLNESVV INCTRPNN NT RKSINIGPGR ALYTTGEIIG DIRQAHCNLS
      CD4 binding
 301  KTQWENTLEQ IAIKLKEQFG NNKTIIFNPS SGGDPEIVTH SFNCGGEFFY
      v4
 351  CNSTQLFTWN DTRKLNNTGR NITLPCRIKQ IINMWQEVGK AMYAPPIRGO
      v5
 401  IRCSSNITGL LLTRDGGKDT NGTEIFRPGG GDMRDNWRSE LYKYKVVKIE
 451  PLGVAPTKAK RRVVQREKR

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- Inner domain
- Bridging sheet
- Outer domain

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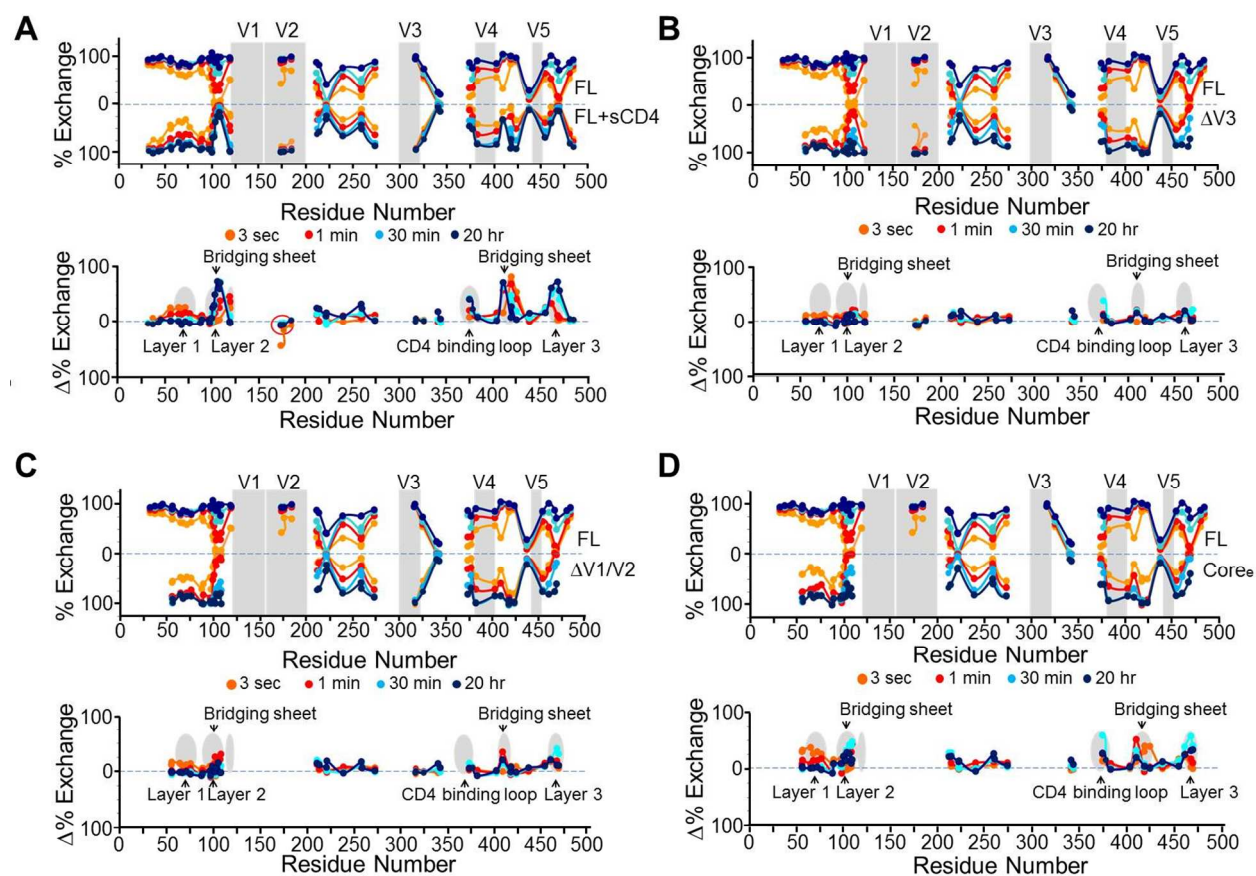
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49 **Figure S3.** Structural dynamics in gp120 monomers deleted with variable loops detected by H/D  
 50 exchange shown in butterfly plots. (A) Butterfly plots for FL (top) and FL+sCD4 (bottom). (B)  
 51 Butterfly plots for FL (top) and  $\Delta V3$  gp120 (bottom). (C) Butterfly plots for FL (top) and  
 52  $\Delta V1/V2$  gp120 (bottom). (D) Butterfly plots for FL (top) and Core<sub>e</sub> gp120 (bottom). The  
 53 exchange is shown by each peptide at the midpoint in primary sequence. The variable loops are  
 54 highlighted in grey. Under each butterfly plot there is a difference plot showing the difference in  
 55 percent exchange at each point. The positions of differences are colored in grey ovals.

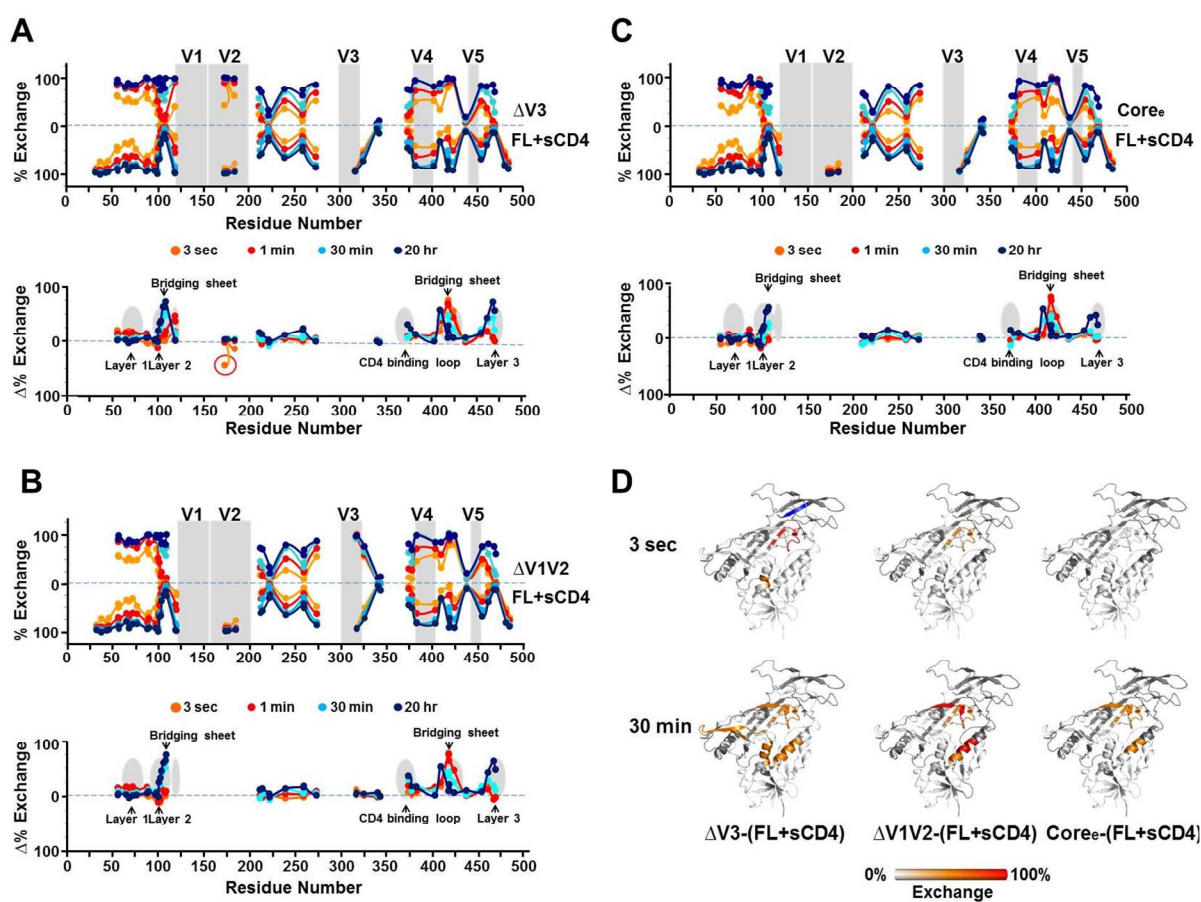


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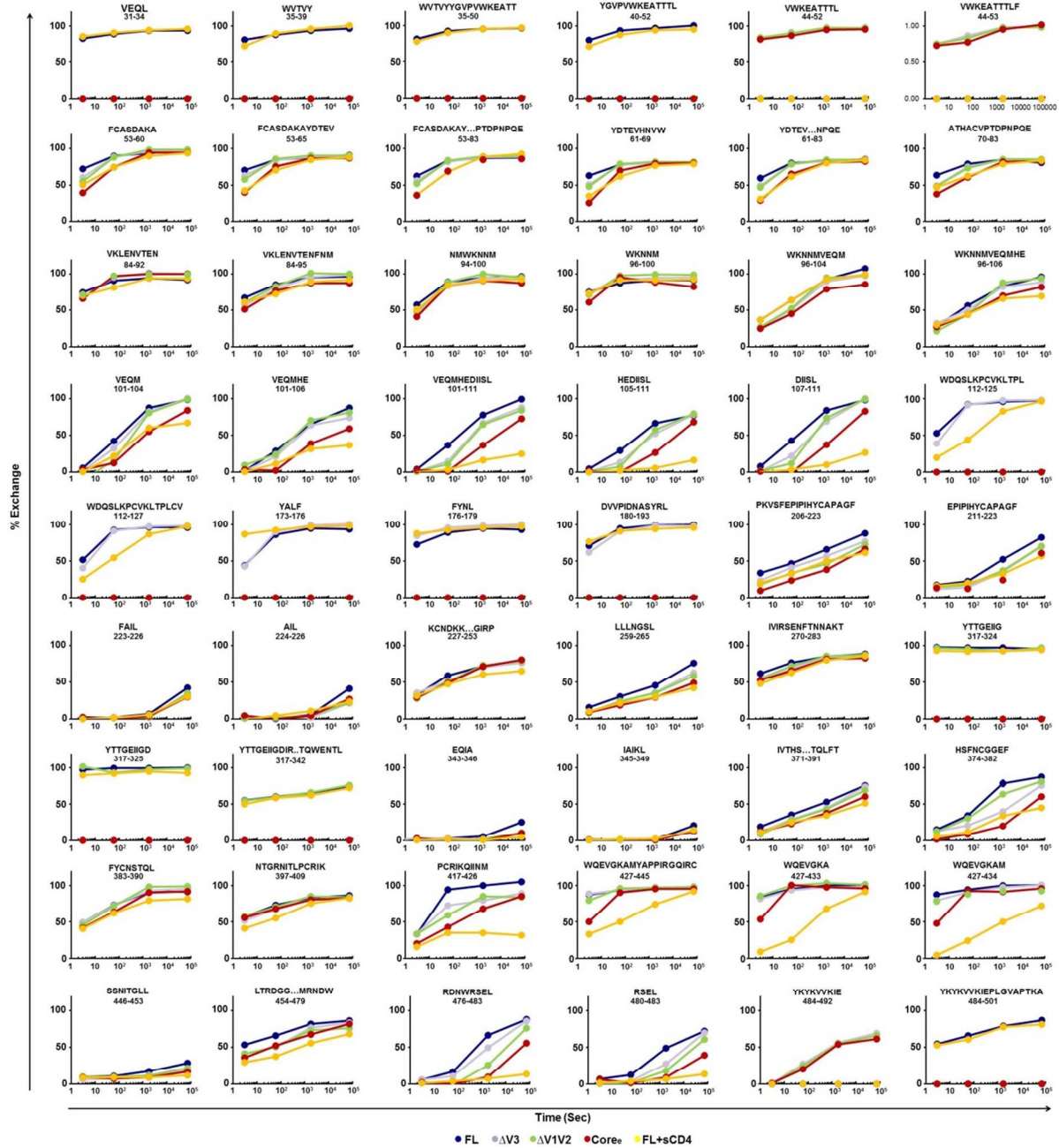
59 **Figure S4.** Structural dynamics in gp120 monomers deleted with variable loops compared to  
60 FL+sCD4 as detected by H/D exchange. (A) Butterfly plots for  $\Delta V3$  (top) and FL+sCD4  
61 (bottom). (B) Butterfly plots for  $\Delta V1/V2$  (top) and FL+sCD4 (bottom). (C) Butterfly plots for  
62 Core<sub>e</sub> (top) and FL+sCD4 (bottom). Explains for the plots were shown in Fig. S3. (D)  
63 Differences in deuterium exchange for  $\Delta V3$  and FL+sCD4,  $\Delta V1/V2$  and FL+sCD4, Core<sub>e</sub> and  
64 FL+sCD4, plotted on the heat map (from left to right). Differences in deuterium exchange at 3  
65 sec were shown in the upper row. Differences in deuterium exchange at 30 min were shown in  
66 the bottom row. The degrees of change between the two proteins are colored from white (<20%  
67 difference) to orange (20%-50% difference) to red (>50% difference).



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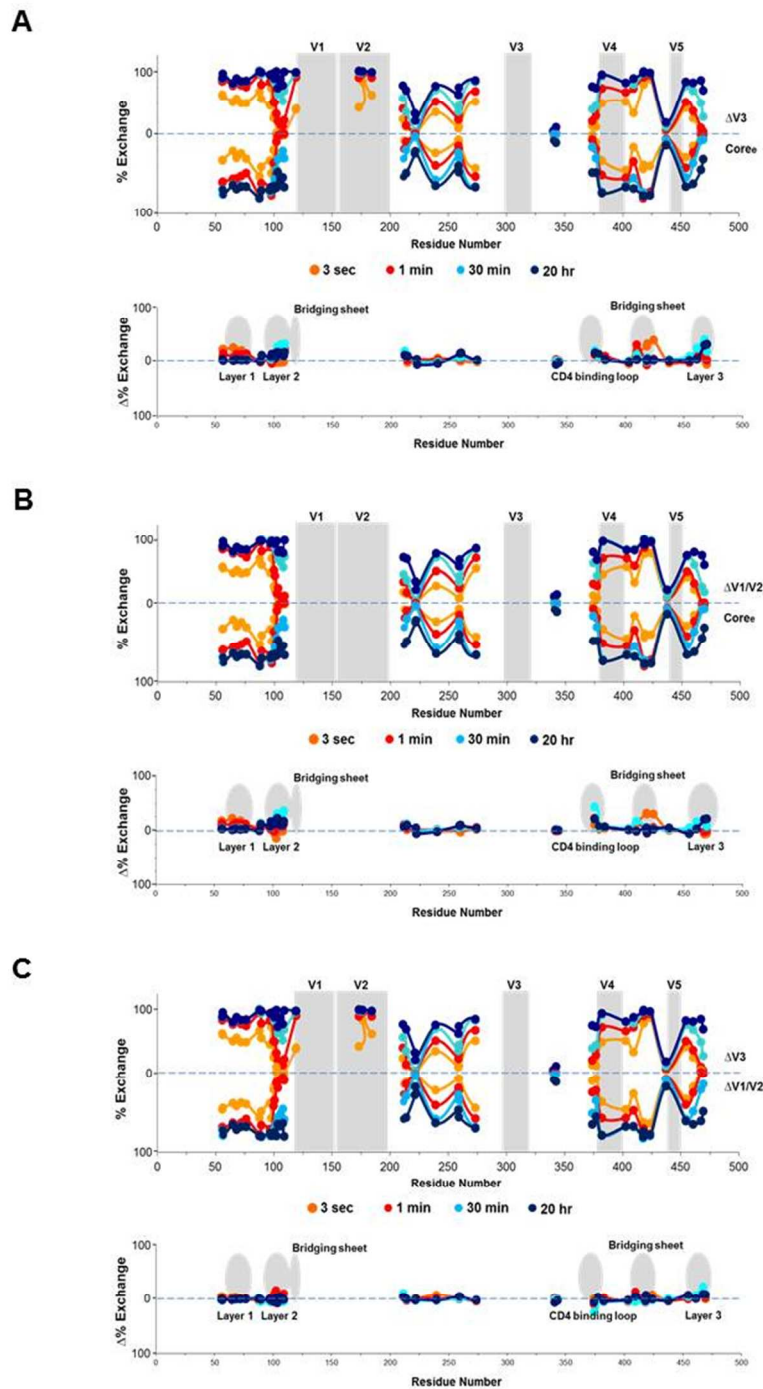
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70 **Figure S5.** Deuterium exchange profiles for the various observable peptides of gp120s.  
 71 Deuterium exchange (%) is shown for monomeric FL (blue),  $\Delta V3$  (purple),  $\Delta V1/V2$  (green),  
 72 Core<sub>e</sub> (red) and FL+sCD4 (yellow) as a function of time (in seconds). Error bars represent  
 73 standard deviations from different observed peptide charge states and duplicate experiments.



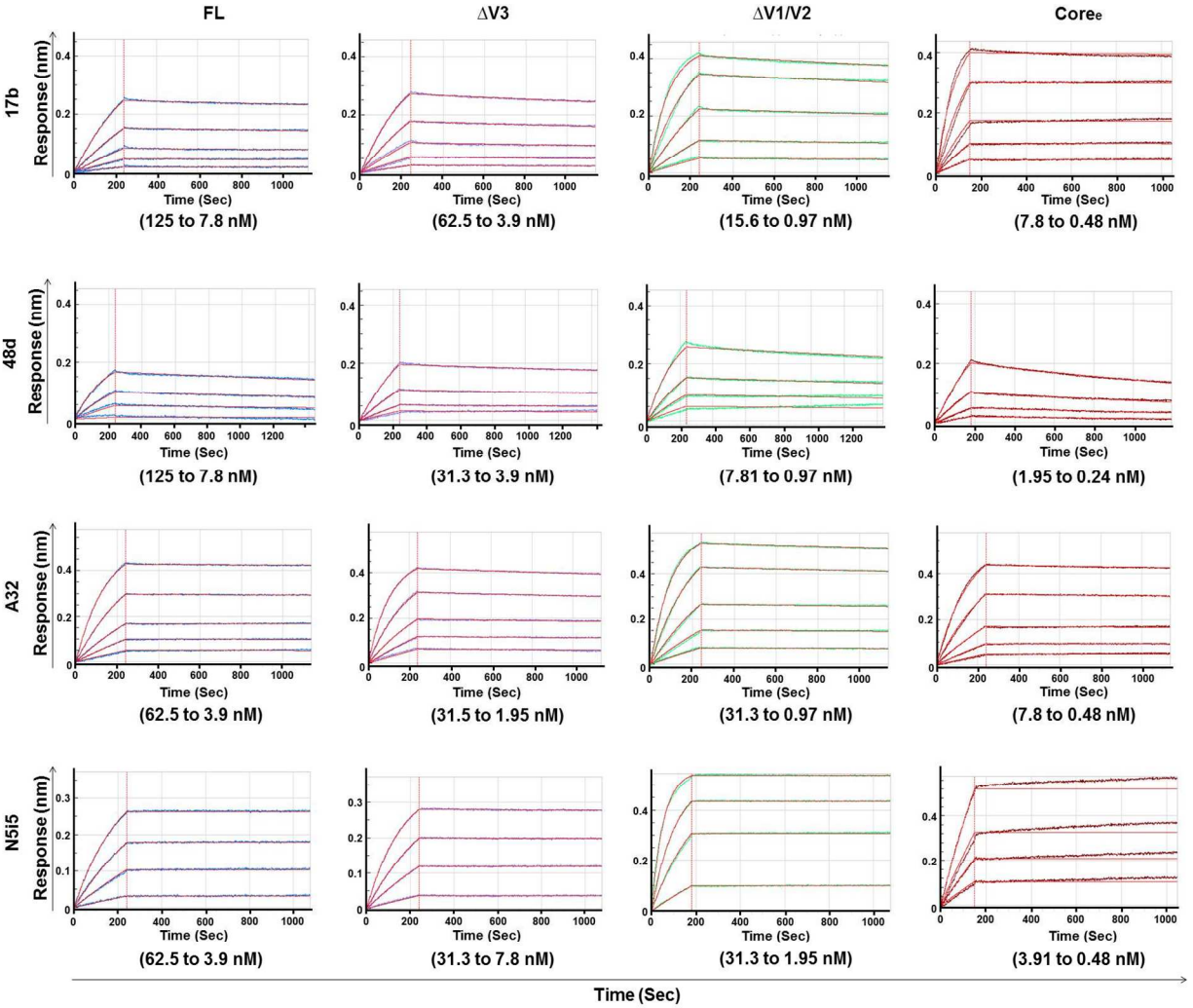
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75 **Figure S6.** Comparisons of H/D exchange profiles of  $\Delta V3$ ,  $\Delta V1/V2$  and  $Core_e$ . (A) Butterfly  
 76 plots for  $\Delta V3$  (top) and  $Core_e$  (bottom). (B) Butterfly plots for  $\Delta V1/V2$  (top) and  $Core_e$  (bottom).  
 77 (C) Butterfly plots for  $\Delta V3$  (top) and  $\Delta V1/V2$  (bottom). Explanations for the butterfly plots were  
 78 referred to Fig. 2.



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80 **Figure S7.** Octet biolayer interferometry binding curves for CD4i binding antibodies. Binding  
 81 curves for FL,  $\Delta V3$ ,  $\Delta V1/V2$  and Core<sub>e</sub> in serial concentrations (indicated under each graph)  
 82 binding to 17b, 48d, A32 and N5i5 captured by AHC biosensors are shown. The binding model  
 83 curves (best-fit 1:1) are colored in red. The four gp120s, FL,  $\Delta V3$ ,  $\Delta V1/V2$  and Core<sub>e</sub> are shown  
 84 in different columns (from left to right). The four antibodies, 17, 48d, A32 and N5i5 are shown  
 85 in different rows (from top to bottom). X-axis is time and Y-axis is binding response (in  
 86 nanometers).

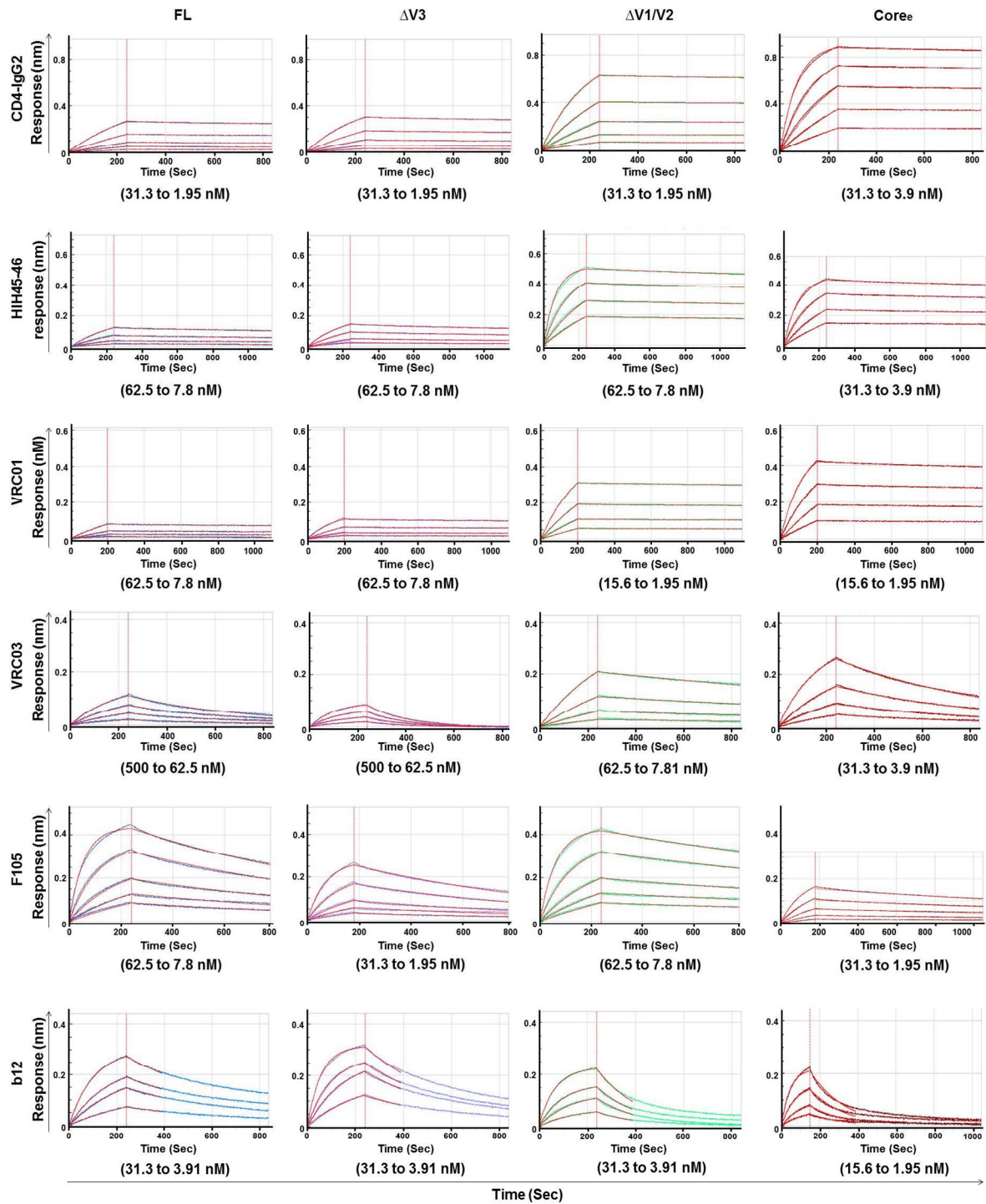


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89 **Figure S8.** Octet biolayer interferometry binding curves for CD4-IgG2 and CD4BS binding  
90 antibodies. Binding curves for FL,  $\Delta V3$ ,  $\Delta V1/V2$  and Core<sub>e</sub> in serial concentrations (indicated  
91 under each graph) binding to CD4-IgG2, NIH45-46, VRC01, VRC03, F105 and b12 captured by  
92 AHC biosensors are shown. The binding model curves (best-fit 1:1) are colored in red. The four  
93 gp120s, FL,  $\Delta V3$ ,  $\Delta V1/V2$  and Core<sub>e</sub> are shown in different columns (from left to right). The  
94 CD4-IgG2, NIH45-46, VRC01, VRC03, F105 and b12 are shown in different rows (from top to  
95 bottom). X-axis is time and Y-axis is binding response (in nanometers).



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