Supplemental Information

Isolate-Specific Differences in the Conformational Dynamics and Antigenicity of HIV-1 gp120

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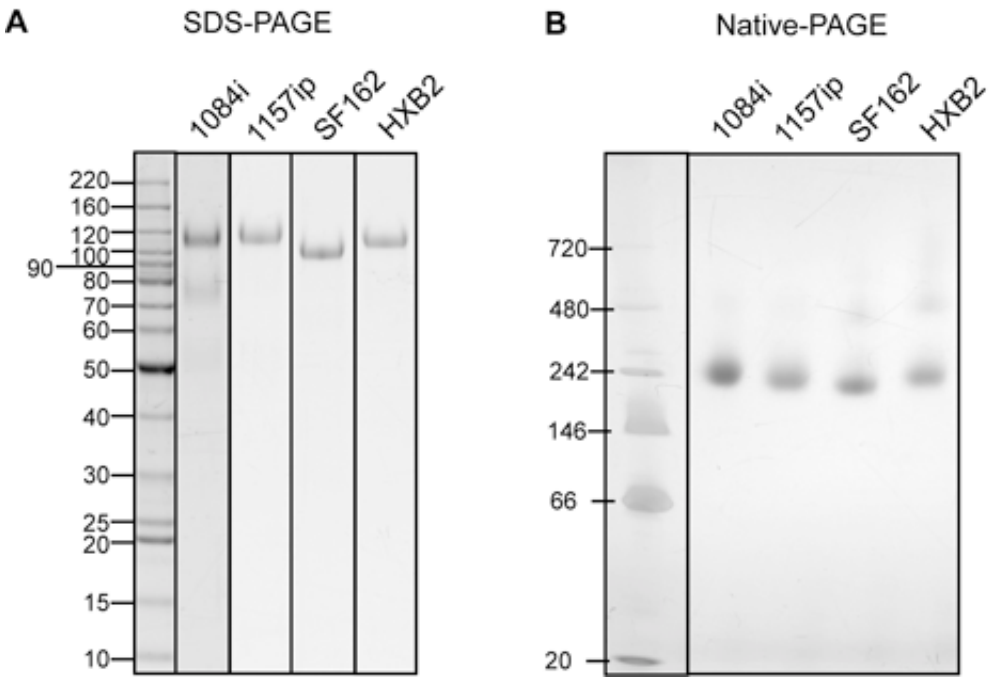

Figure S1. SDS- and Native-PAGE analysis of purified gp120 proteins. (A) SDS-PAGE analysis of purified gp120 proteins. (B) Blue Native-PAGE analysis of purified monomeric gp120.

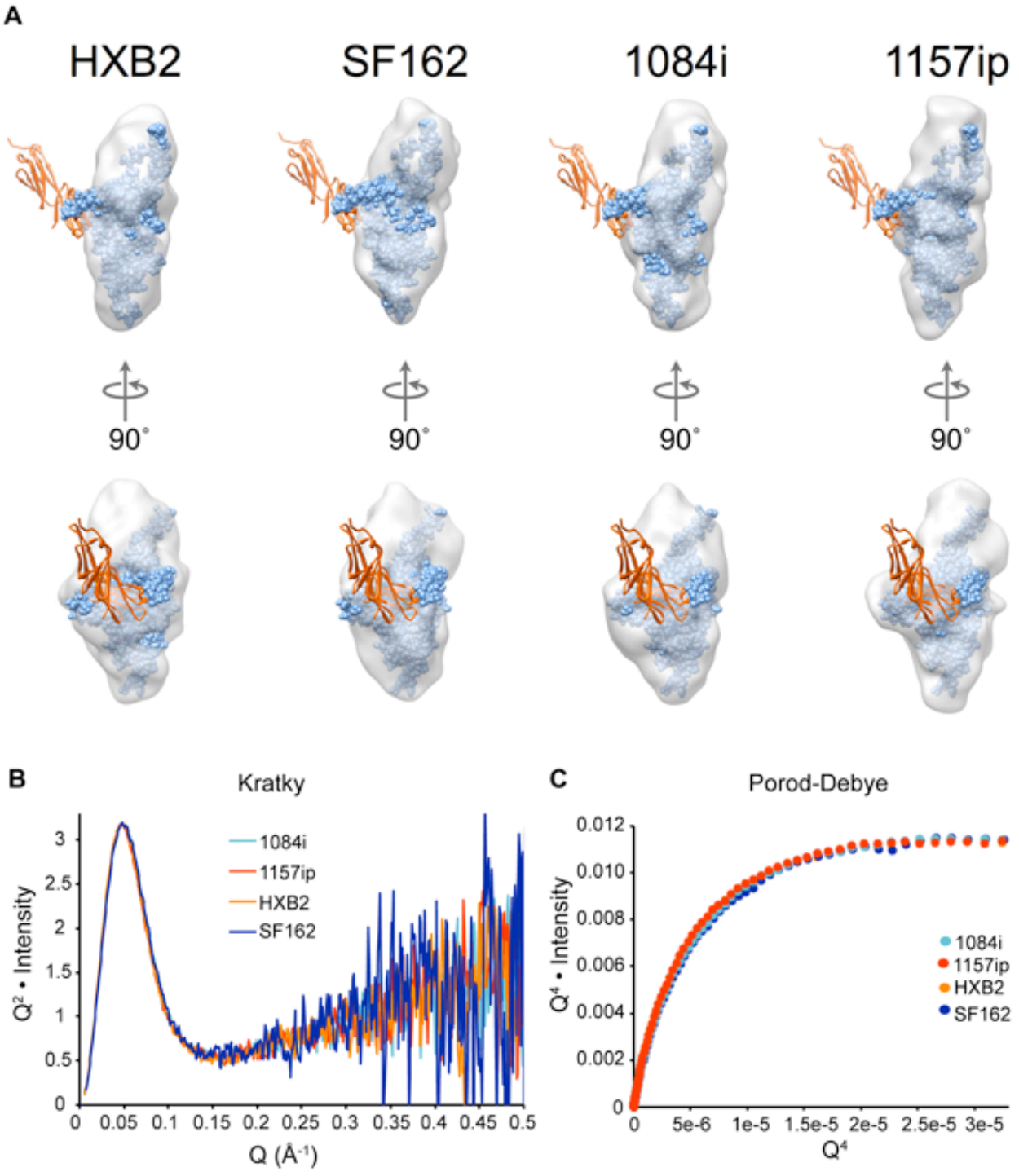

Figure S2. SAXS reconstruction, Kratky plot, and Porod-Debye analysis of SAXS data. **(A)** DAMMIN reconstructions of the four full-length gp120 monomers (gray density) overlayed with a space-filling model of the gp120 core (3JWD) including the V3 loop from (2B4C) (blue spheres) (B) Kratky and (C) Porod-Debye analysis of SAXS data. Each isolate is represented by a different color, as shown in the legend. The data are superimposable for all for gp120s, indicating similar degrees of globular structure.

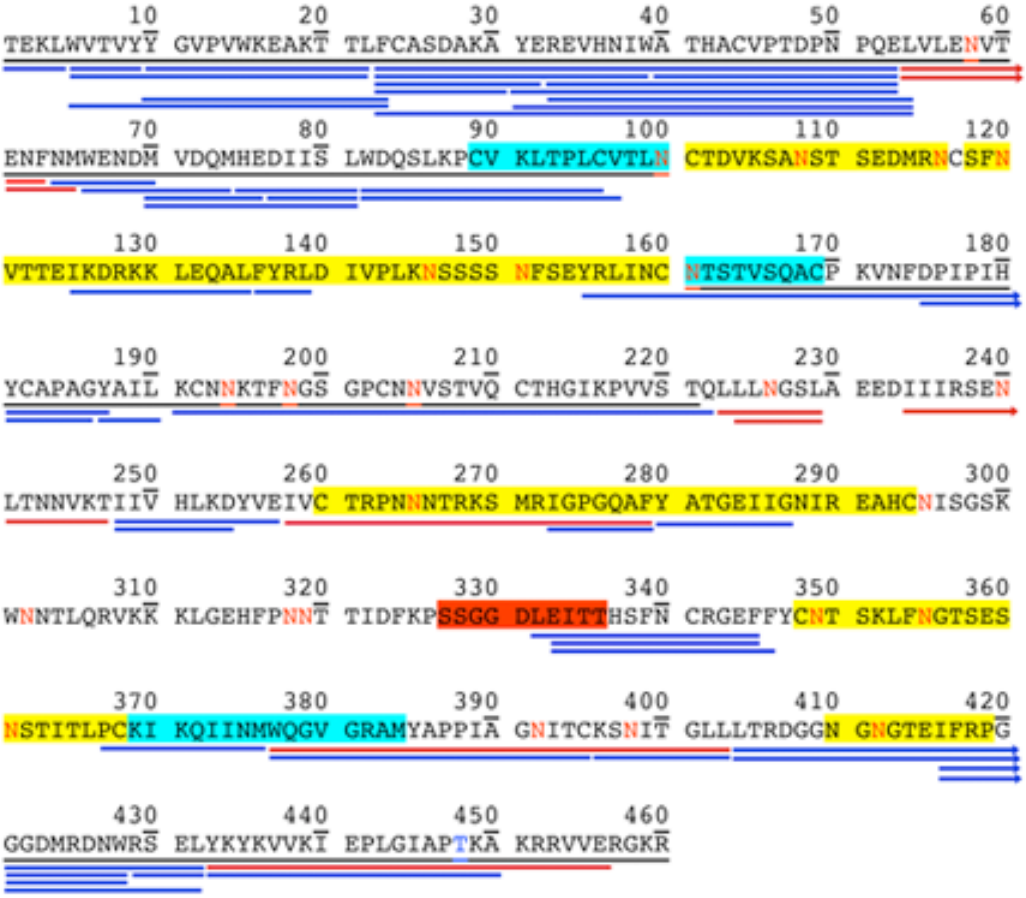

Figure S3. Pepsin digestion map for 1084i gp120. The mature 1084i gp120 amino sequence is indicated (with numbering based on mature 1084i). Inner domain elements are underlined (thin black line), variable loops are indicated in yellow, bridging sheet elements highlighted in cyan, and CD4 binding loop in red. Red text indicates sites of PNGS, blue text indicates O-linked glycosylation site. Blue and red lines beneath the sequence indicate peptides and glycopeptides, respectively, that are observable in HDX-MS analysis.

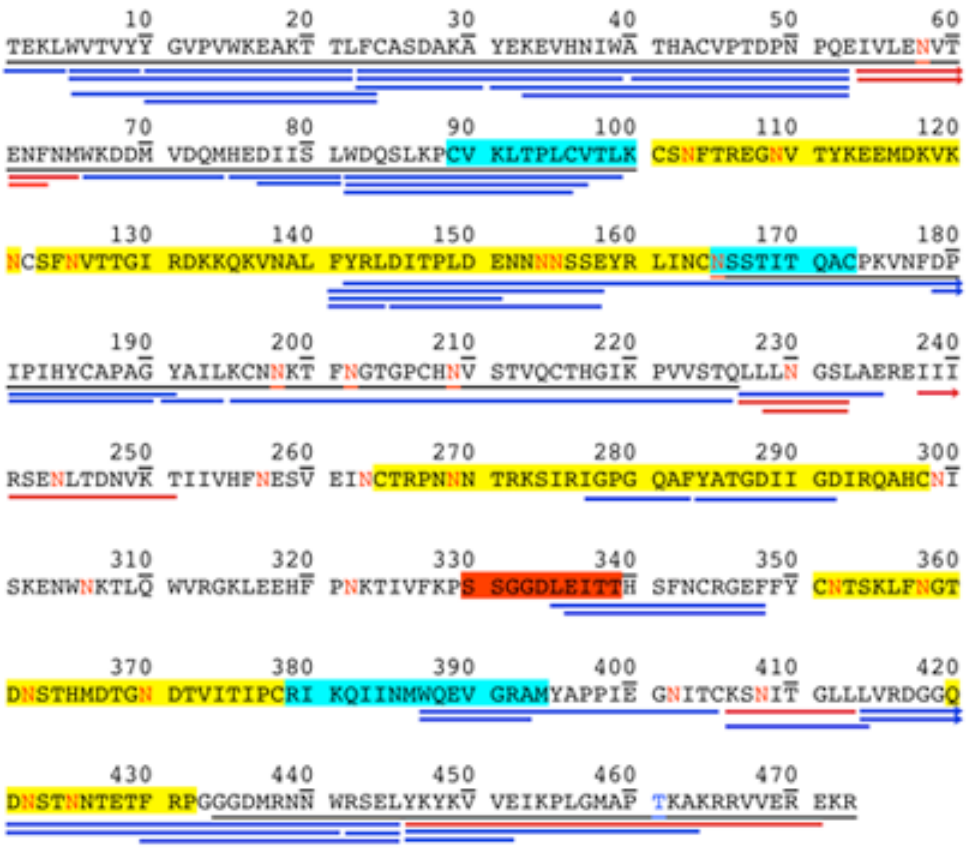

Figure S4. Pepsin digestion map for 1157ip gp120. The mature 1157ip gp120 amino sequence is indicated (with numbering based on mature 1157ip). Inner domain elements are underlined (thin black line), variable loops are indicated in yellow, bridging sheet elements highlighted in cyan, and CD4 binding loop in red. Red text indicates sites of PNGS, blue text indicates O-linked glycosylation site. Blue and red lines beneath the sequence indicate peptides and glycopeptides, respectively, that are observable in HDX-MS analysis.

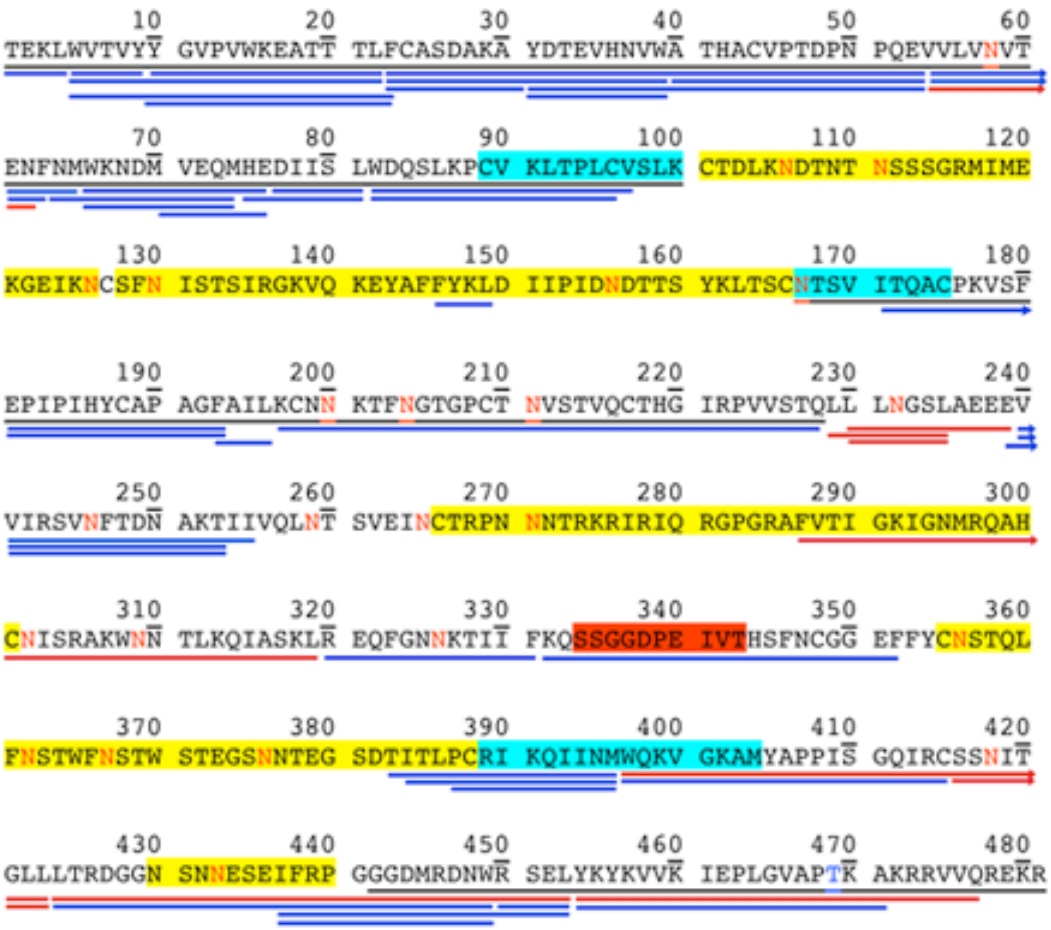

Figure S5. Pepsin digestion map for HXB2 gp120. The mature HXB2 gp120 amino sequence is indicated (with numbering based on mature HXB2). Inner domain elements are underlined (thin black line), variable loops are indicated in yellow, bridging sheet elements highlighted in cyan, and CD4 binding loop in red. Red text indicates sites of PNGS, blue text indicates O-linked glycosylation site. Blue and red lines beneath the sequence indicate peptides and glycopeptides, respectively, that are observable in HDX-MS analysis.

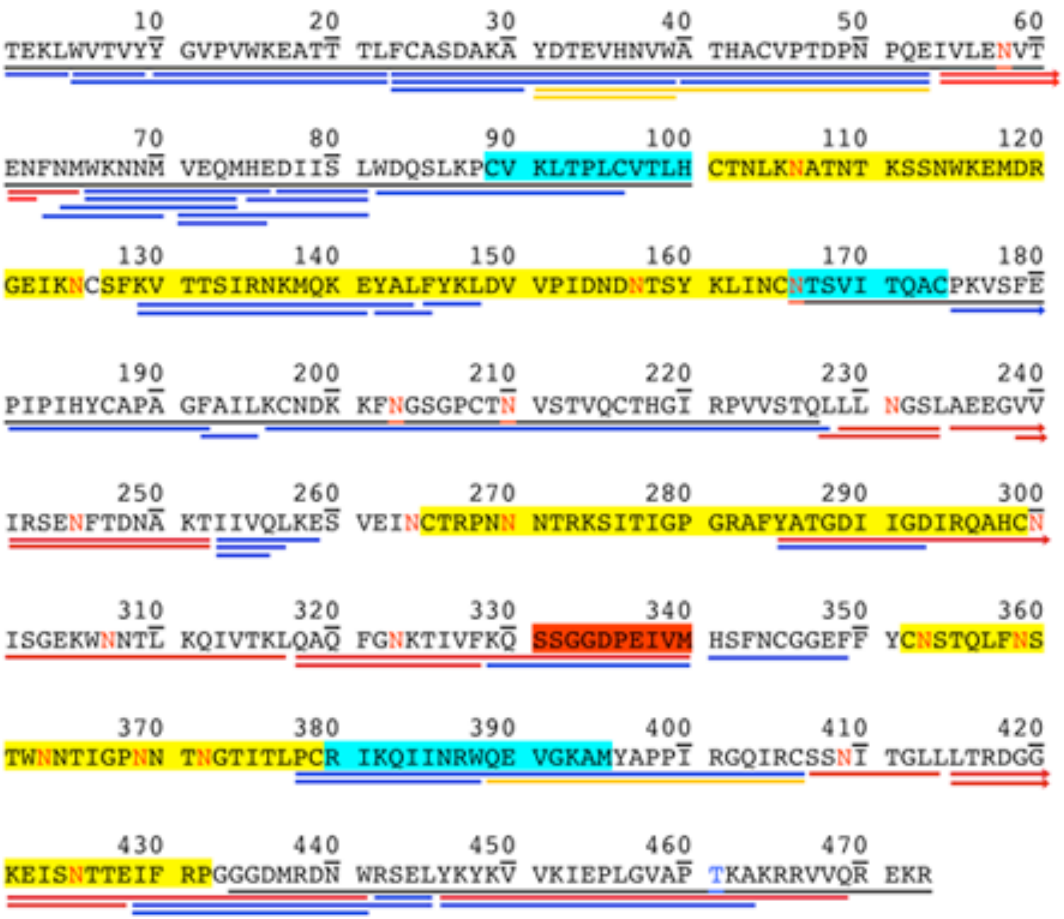

Figure S6. Pepsin digestion map for SF162 gp120. The mature SF162 gp120 amino sequence is indicated (with numbering based on mature SF162). Inner domain elements are underlined (thin black line), variable loops are indicated in yellow, bridging sheet elements highlighted in cyan, and CD4 binding loop in red. Red text indicates sites of PNGS, blue text indicates O-linked glycosylation site. Blue and red lines beneath the sequence indicate peptides and glycopeptides, respectively, that are observable in HDX-MS analysis. Yellow lines indicate coverage by subtraction of overlapping peptides.

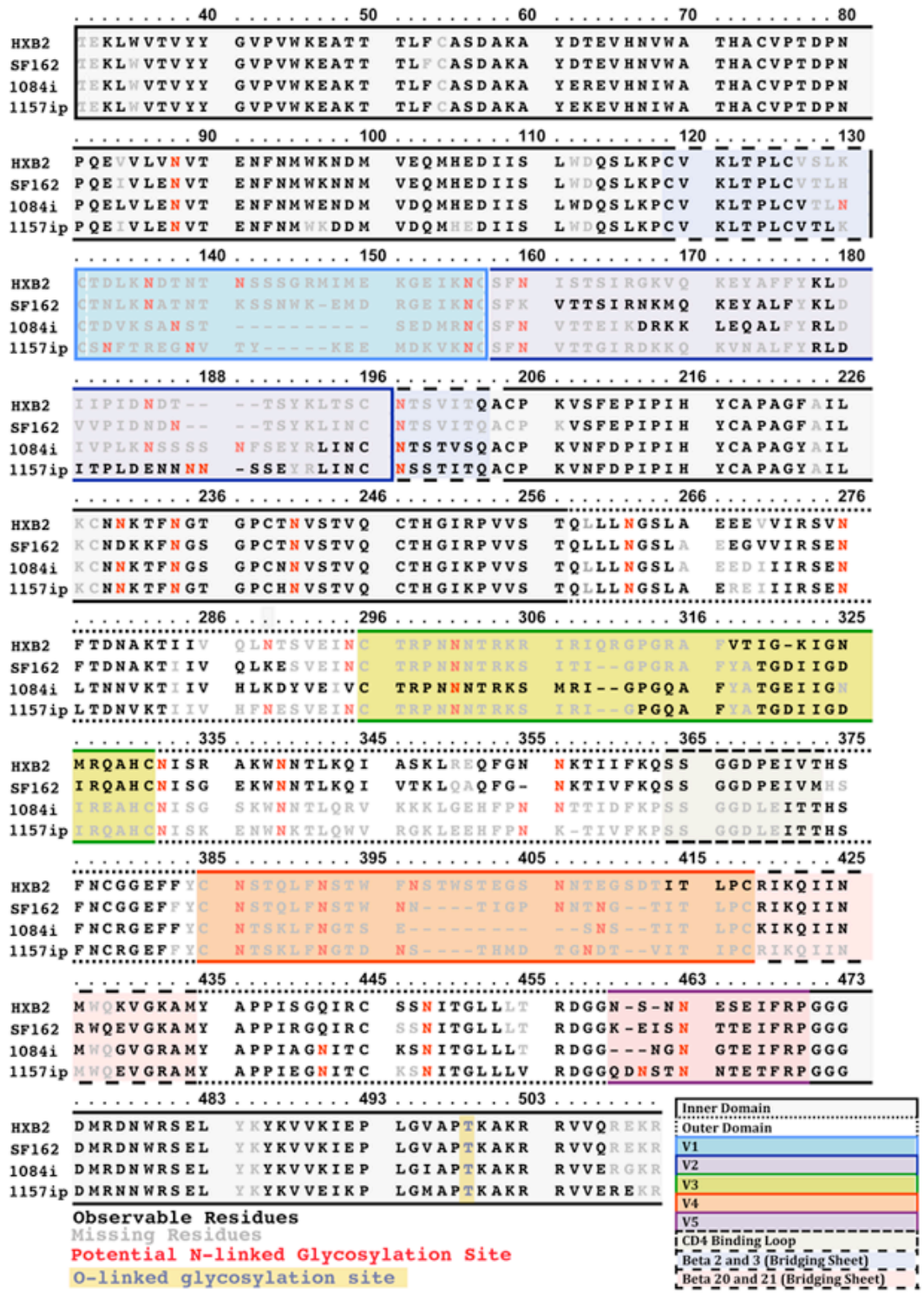

Figure S7. Sequence coverage of all peptides for every isolate. The four isolates are aligned to HXB2 and numbered based on standard HXB2 numbering. HDX-MS-observable residues are indicated in black; residues that cannot be measured as a result of missing peptide coverage or rapid back-exchange (N-terminal residues of peptic fragments) are indicated in grey. Variable loops, bridging sheet elements, and CD4-binding loop are colored as described in the inset.

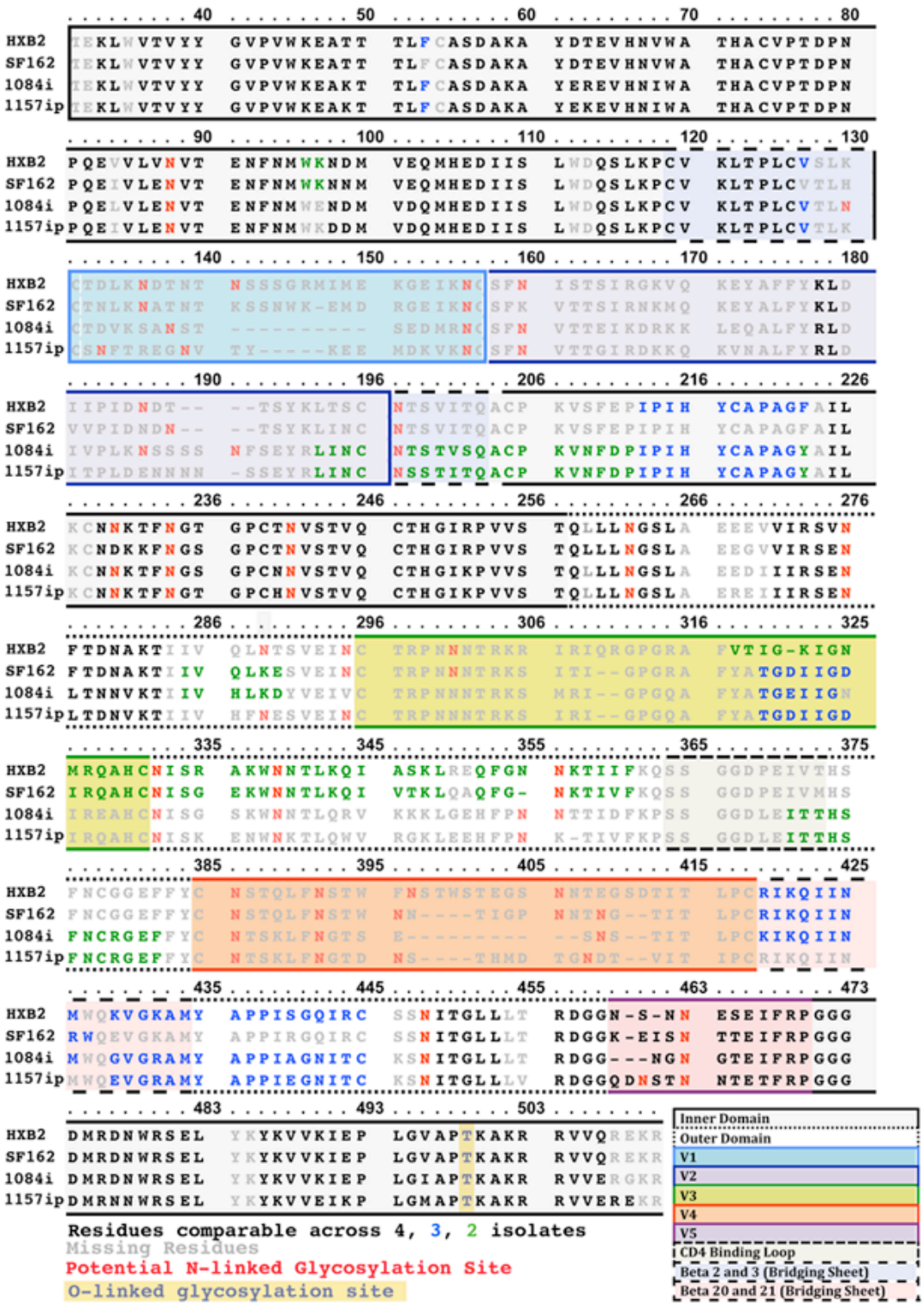

Figure S8. Sequence coverage of peptides that are comparable across isolates. The four isolates are aligned to HXB2 and numbered based on standard HXB2 numbering. Black, blue and green text indicates residues that are comparable across 4, 3 and 2 isolates, respectively; residues that cannot be compared across isolates as a result of missing peptide coverage or rapid back-exchange (N-terminal residues of peptic fragments) are indicated in grey. Variable loops, bridging sheet elements, and CD4-binding loop are colored as described in the inset.

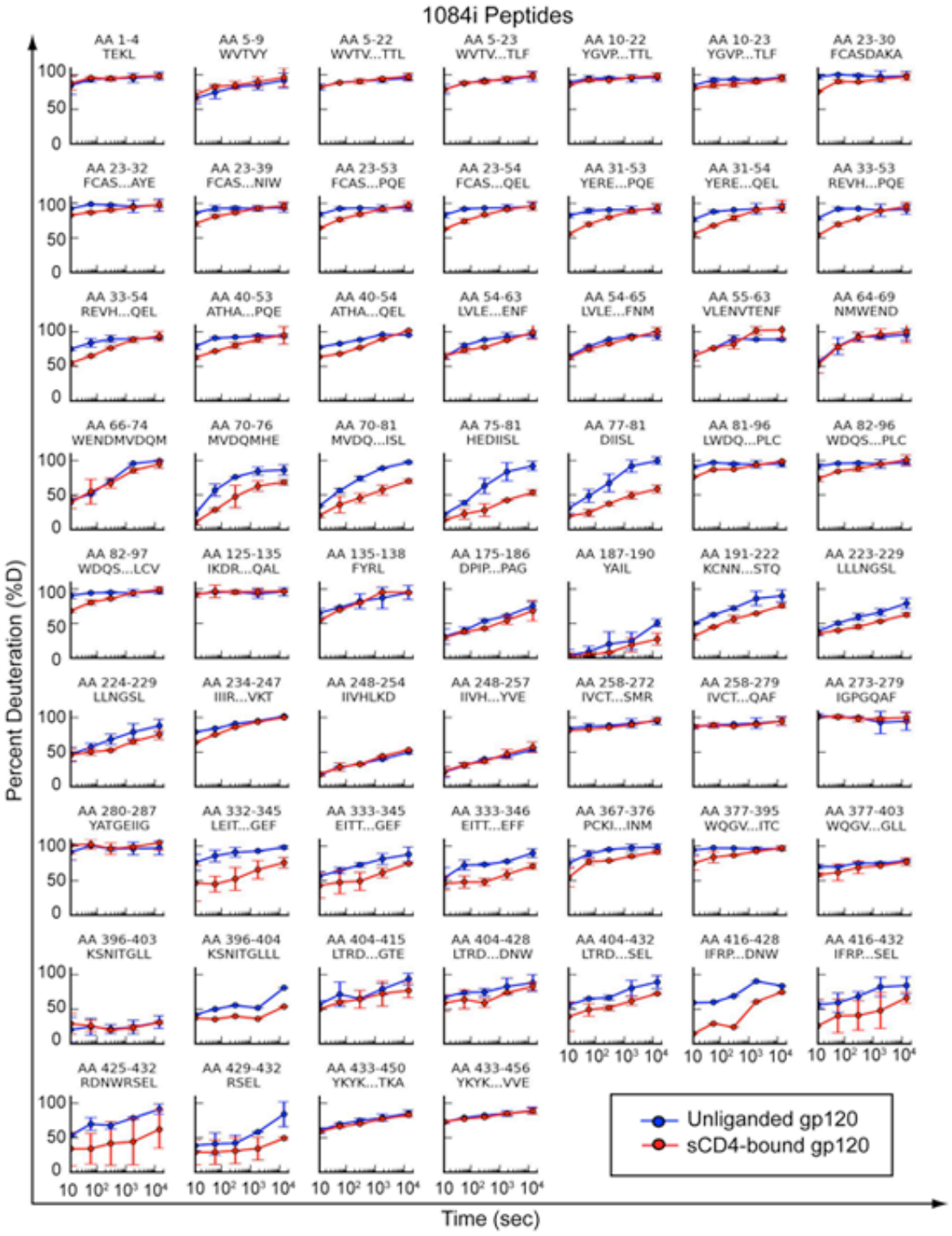

Figure S9. Deuteration plots of all unique 1084i peptides (+/-) sCD4. Deuterium uptake plots with percent deuteration vs time are shown for each unique peptide observed for 1084i gp120. Data for unliganded gp120 is shown in blue, while data for sCD4-bound gp120 is shown in red. Amino acid sequence and position of each peptide (using the same numbering as in Figure S3) are indicated above each plot.

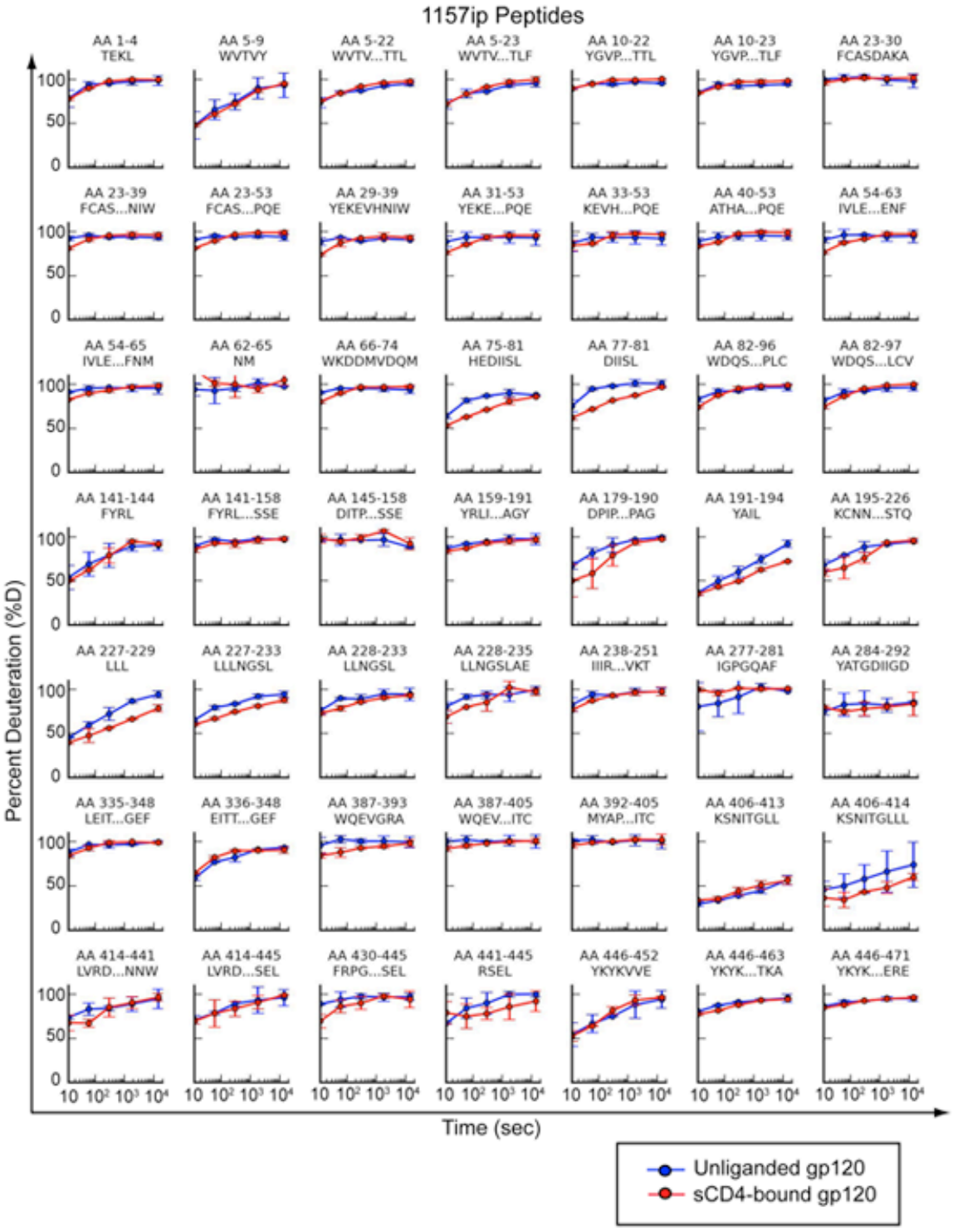

Figure S10. Deuteration plots of all unique 1157ip peptides (+/-) sCD4. Deuterium uptake plots with percent deuteration vs. time are shown for each unique peptide observed for 1157ip gp120. Data for unliganded gp120 is shown in blue, while data for sCD4-bound gp120 is shown in red. Amino acid sequence and position of each peptide (using the same numbering as in Figure S4) are indicated above each plot.

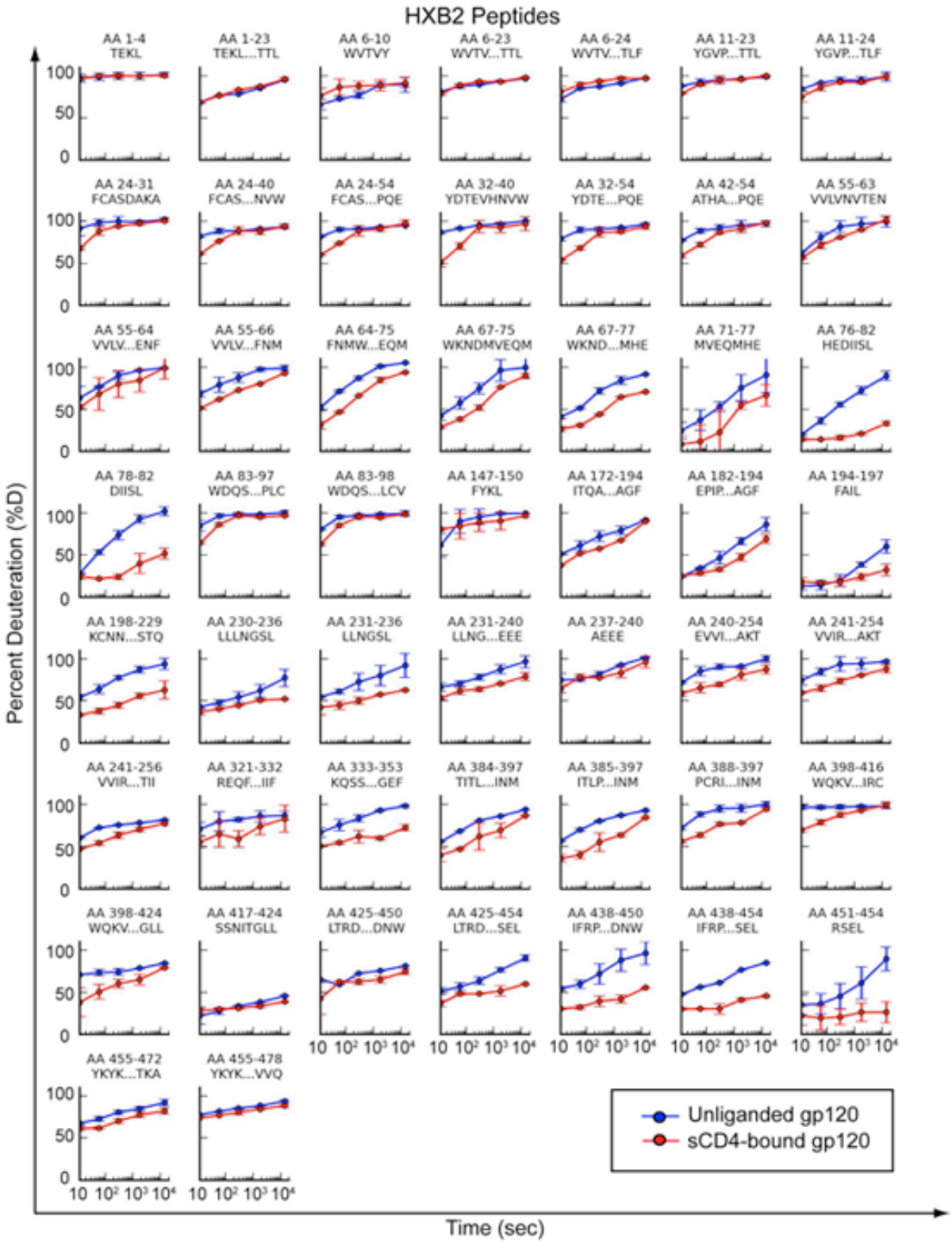

Figure S11. Deuteration plots of all unique HXB2 peptides (+/-) sCD4. Deuterium uptake plots with percent deuteration over time are shown for each unique peptide observed for HXB2 gp120. Data for unliganded gp120 is shown in blue, while data for sCD4-bound gp120 is shown in red. Amino acid sequence and position of each peptide (using the same numbering scheme as in Figure S5) are indicated above each plot.

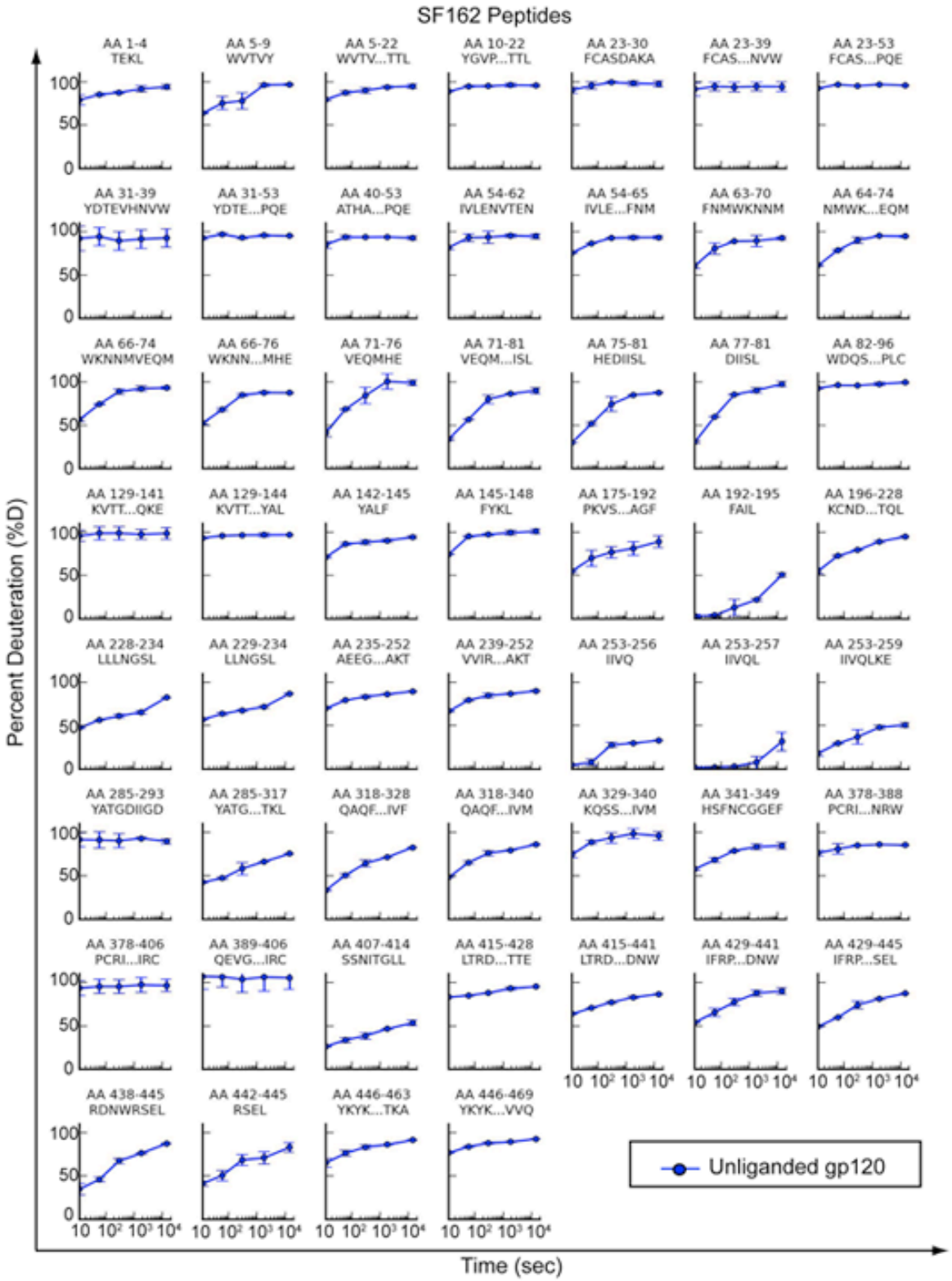

Figure S12. Deuteration plots of all unique SF162 peptides. Deuterium uptake plots with percent deuteration over time are shown for each unique peptide observed for SF162 gp120. Data for unliganded gp120 is shown in blue, data was not collected for sCD4-bound SF162, as it has been previously characterized. Amino acid sequence and position of each peptide (using the same numbering scheme as in Figure S6) are indicated above each plot.

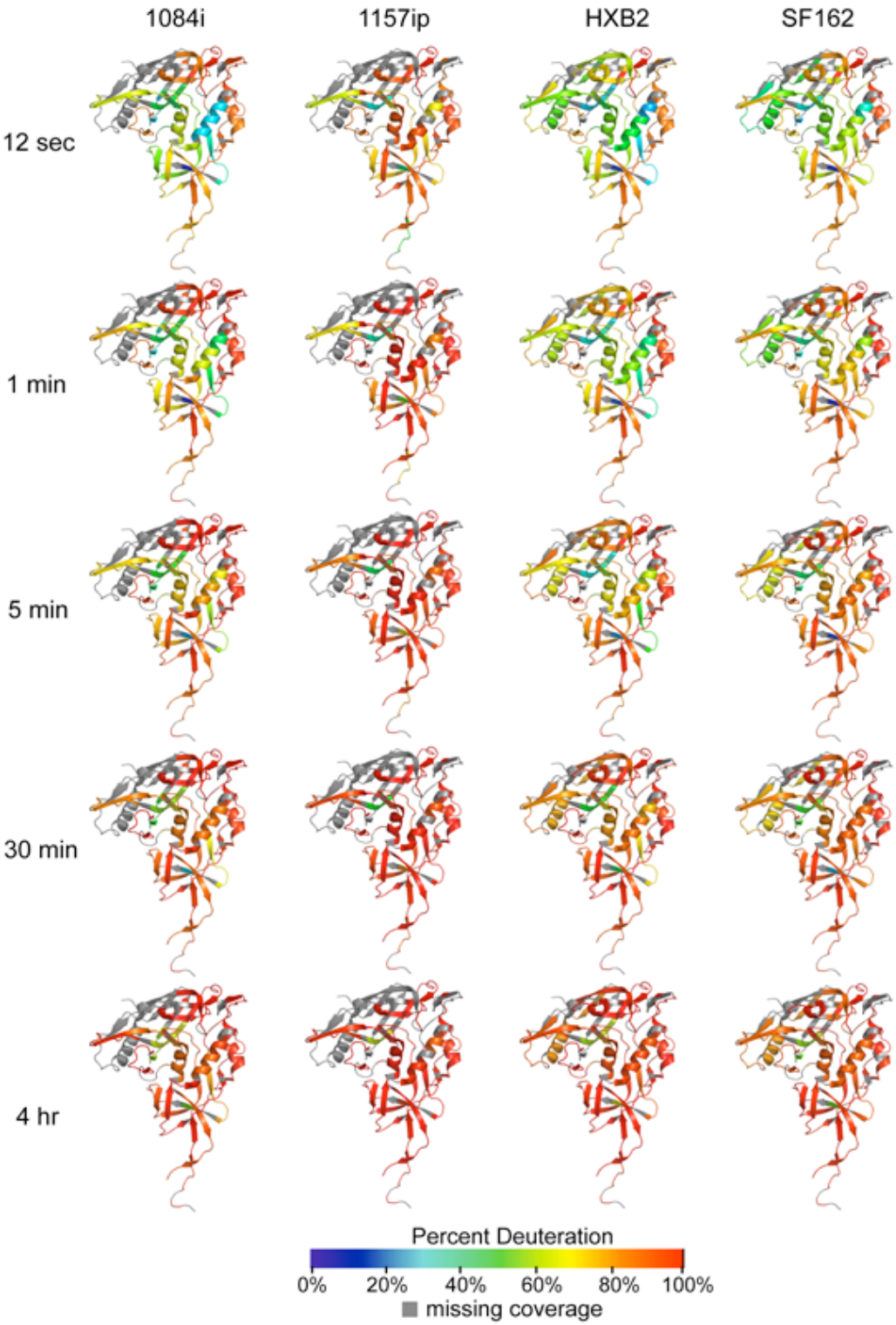

Figure S13. Heat map summary of HDX-MS data. Colors mapped onto gp120 core (PDB ID: 3JWD) indicate percent deuteration of peptides throughout gp120 at each deuteration time point (12 sec, 1 min, 5 min, 30 min, 4 hr) for each of the four unliganded gp120 proteins. Deuteration levels are indicated in the legend, with red colors corresponding to high levels of deuterium uptake (dynamic regions) and blue colors corresponding to low levels of deuteration (ordered or "protected" regions). Regions where peptide information is missing are indicated in gray.

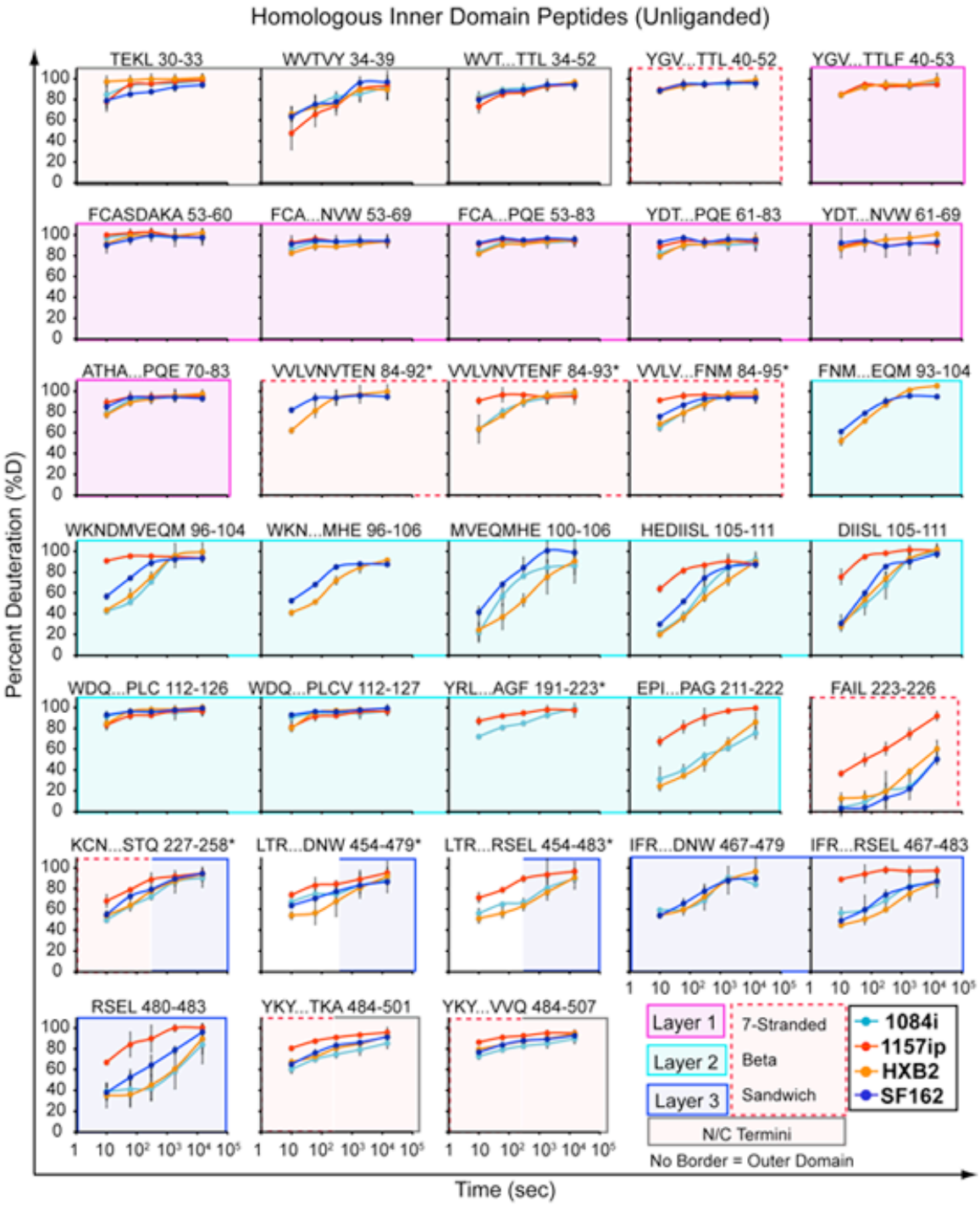

Figure S14. Deuteration plots for all comparable inner domain peptides (unliganded gp120). Each graph shows deuterium uptake (% deuteration) over time for peptides throughout the gp120 inner domain, which are either identical or homologous among at least two gp120 isolates. The peptide sequence and amino acid position (both according to HXB2) are indicated for each graph. Each line on the graph corresponds to the deuterium uptake for a different isolate, as indicated in the figure legend. The position of each peptide in the context of the gp120 inner domain is indicated by the colored boxes surrounding the graphs, as defined in the figure legend. Half-boxes indicate peptides that occupy a sequence spanning two separate regions. (*) indicates glycopeptides.

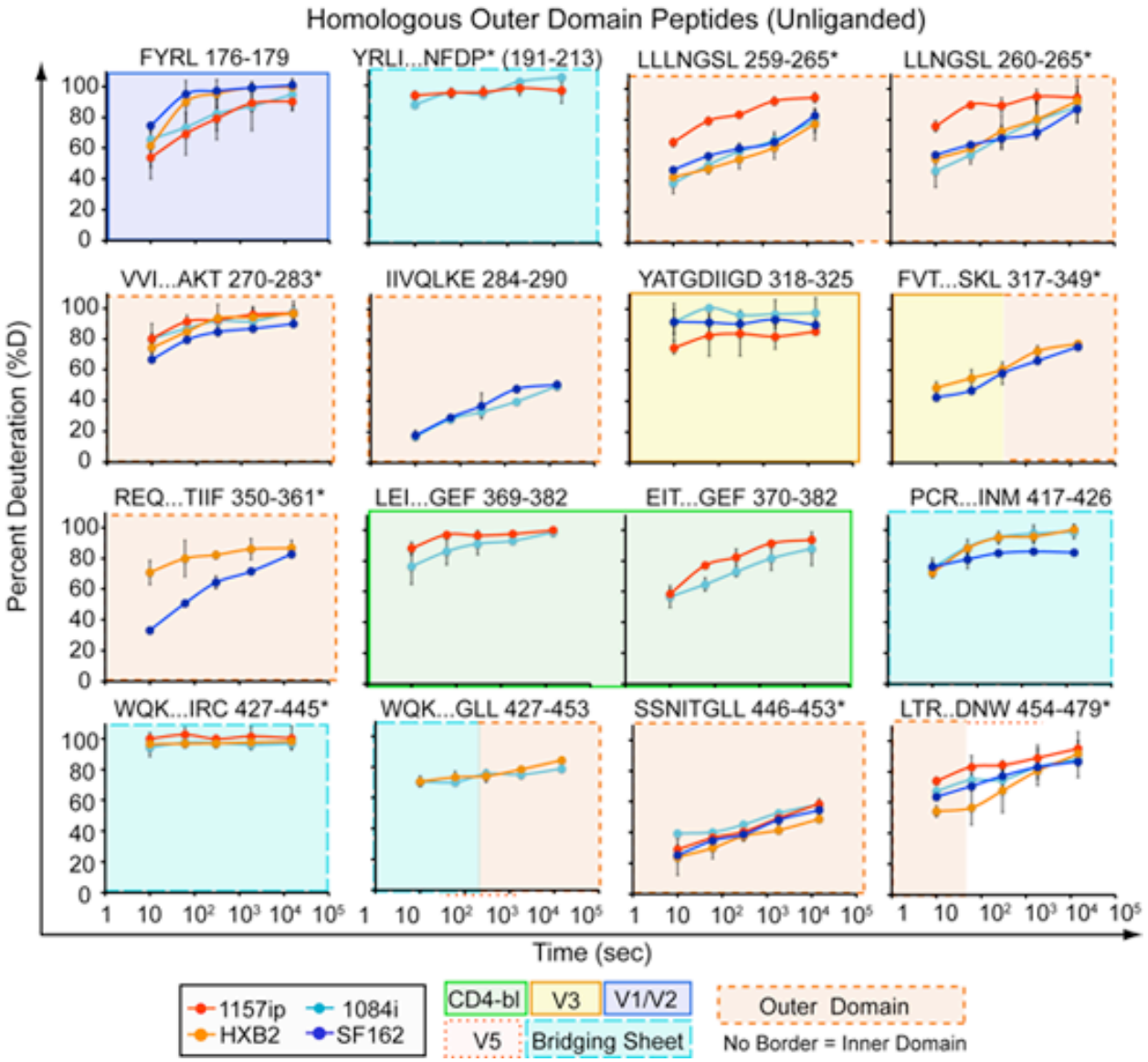

Figure S15. Deuteration plots for all comparable outer domain/variable loop/bridging sheet peptides (unliganded gp120). Each graph shows deuterium uptake (% deuteration) over time for peptides throughout the gp120 outer domain, variable loops, or bridging sheet, which are either identical or homologous among at least two gp120 isolates. The peptide sequence and amino acid position (both according to HXB2, unless a peptide for HXB2 was not observed) are indicated for each graph. Each line on the graph corresponds to the deuterium uptake for a different isolate, as indicated in the figure legend. The position of each peptide in the context of the gp120 inner domain is indicated by the colored boxes surrounding the graphs, as defined in the figure legend. Partial-boxes indicate peptides that occupy a sequence spanning two separate regions. (*) indicates glycopeptides.

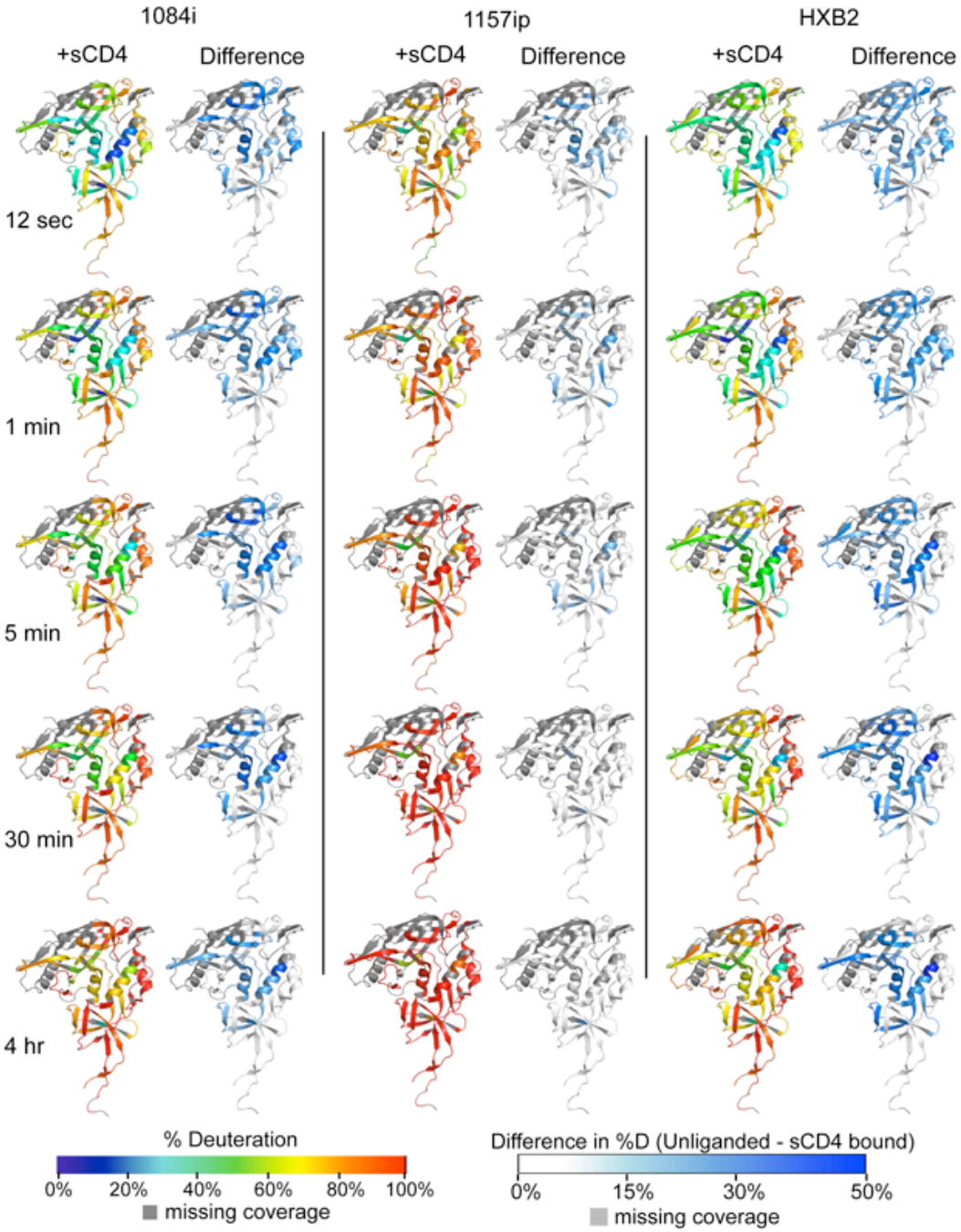

Figure S16. Heat map summary of sCD4-bound gp120 deuteration and difference maps. Data for each gp120 isolate (1084i, 1157ip, and HXB2) is presented in a separate column, and each row reflects a different time point (12 sec, 1 min, 5 min, 30 min, and 4 hr). Within each column, the colors mapped onto gp120 core (PDB ID: 3JWD) indicate percent deuteration (left) or difference in percent deuteration relative to unliganded gp120 (right). Deuteration levels are indicated in the legend on the left, with red colors corresponding to high levels of deuterium uptake (dynamic regions) and blue colors corresponding to low levels of deuteration (ordered or "protected" regions). Regions where peptide information is missing are indicated in gray. Deuteration difference values are indicated in the legend on the right, where white colors reflect regions that are similarly deuterated in unliganded and sCD4-bound gp120 and blue colors reflect regions that are stabilized as a result of sCD4-binding.

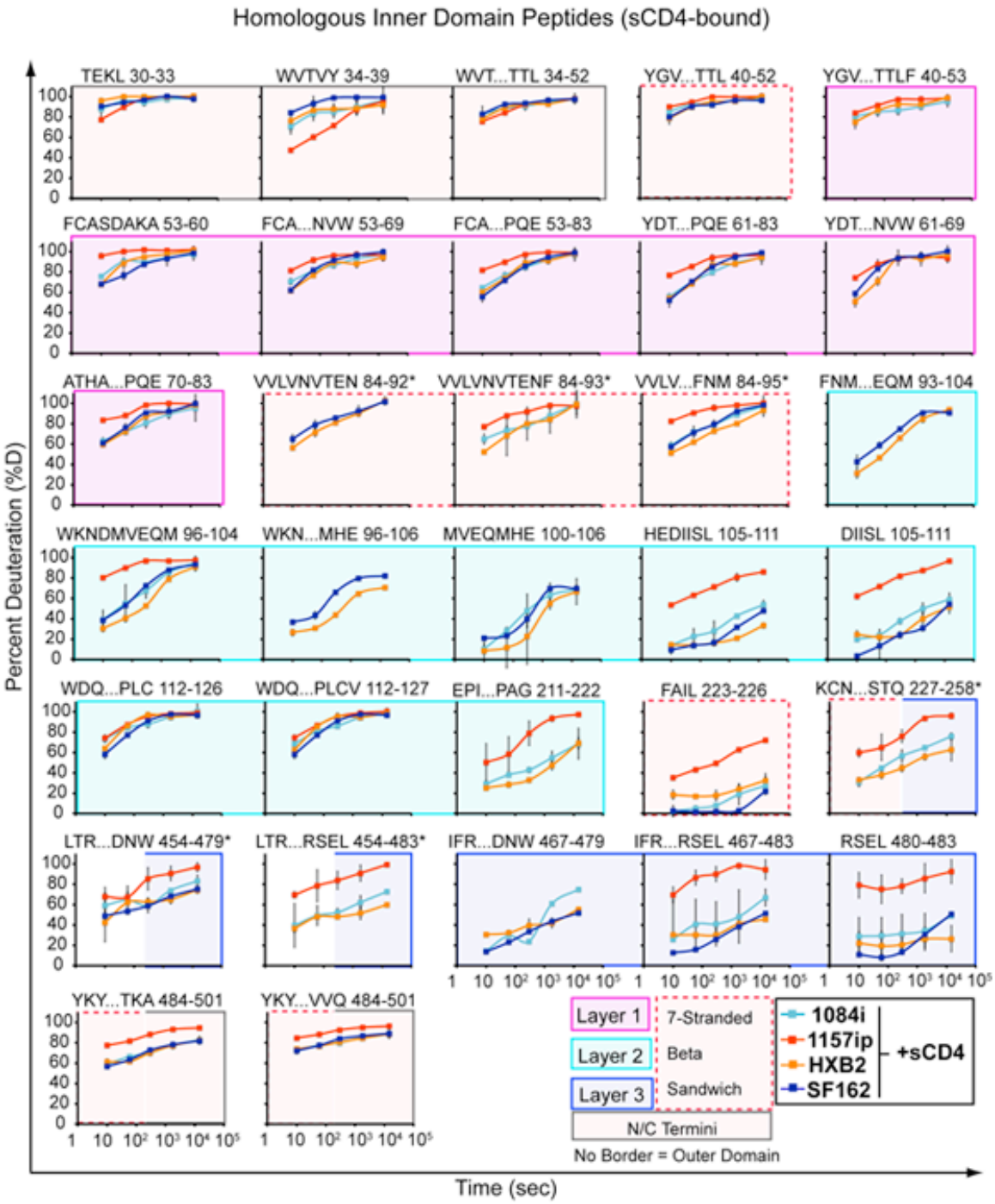

Figure S17. Deuteration plots for all comparable inner domain peptides (sCD4-bound gp120). Each graph shows deuterium uptake (% deuteration) over time for peptides throughout the gp120 inner domain in sCD4-bound gp120, which are either identical or homologous among at least two gp120 isolates. The peptide sequence and amino acid position (both according to HXB2, unless a peptide for HXB2 was not observed) are indicated for each graph. Each line on the graph corresponds to the deuterium uptake for a different isolate, as indicated in the figure legend. The position of each peptide in the context of the gp120 inner domain is indicated by the colored boxes surrounding the graphs, as defined in the figure legend. Half-boxes indicate peptides that occupy a sequence spanning two separate regions. (*) indicates glycopeptides.

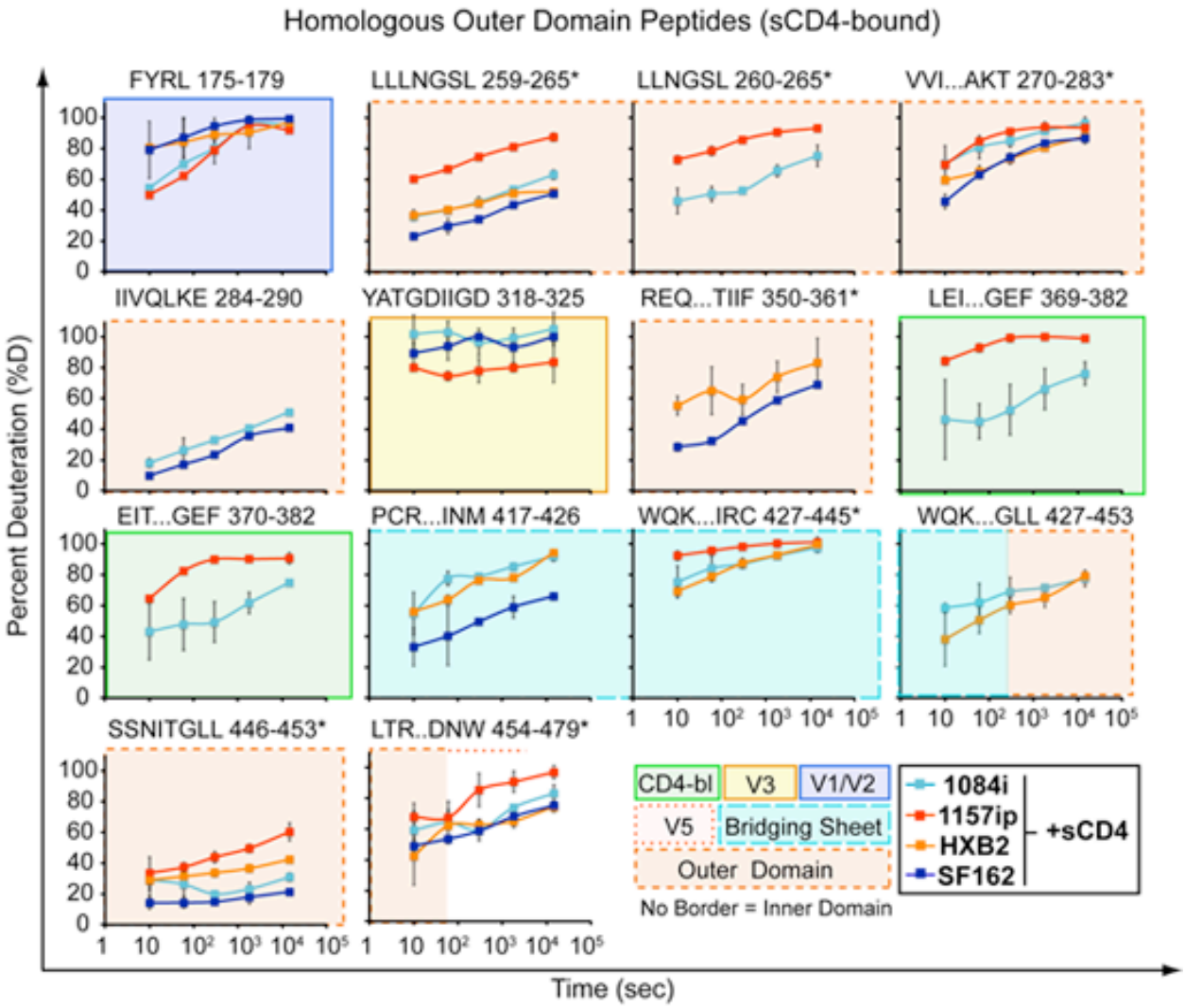

Figure S18. Deuteration plots for all comparable outer domain/variable loop/bridging sheet peptides (sCD4-bound gp120) Each graph shows deuterium uptake (% deuteration) over time for peptides throughout the gp120 outer domain, variable loops or bridging sheet, which are either identical or homologous among at least two gp120 isolates. The peptide sequence and amino acid position (both according to HXB2) are indicated for each graph. Each line on the graph corresponds to the deuterium uptake for a different isolate, as indicated in the figure legend. The position of each peptide in the context of the gp120 inner domain is described by the colored boxes surrounding the graphs, as defined in the figure legend. Partial-boxes indicate peptides that occupy a sequence spanning two separate regions. (*) indicates glycopeptides.

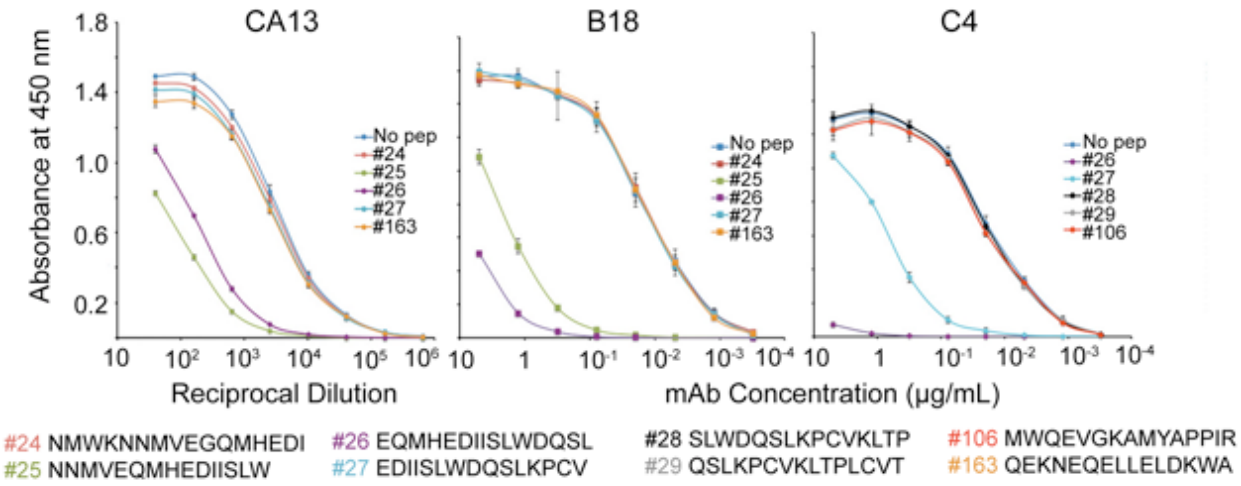

Figure S19. Linear epitope-specific antibody peptide competition ELISA. Antibody binding curves are shown for mAbs CA13, C4, and B18 binding to SF162 gp120 in the presence or absence of competing peptides. Peptide competition for antibody binding to gp120 is reflected by a shift in the binding curve to lower levels relative to the "no pep" control. For each antibody binding curve, the different lines indicate antibody binding in the presence of a different peptide as described in the inset for each binding curve. The x-axis for CA13 shows reciprocal dilution of antibody supernatant, and the x-axis for the B18 and C4 curves shows monoclonal antibody concentration. The identity of each peptide number is defined below the graphs. Axes show antibody binding levels (measured as absorbance at 450 nm) vs antibody concentration.

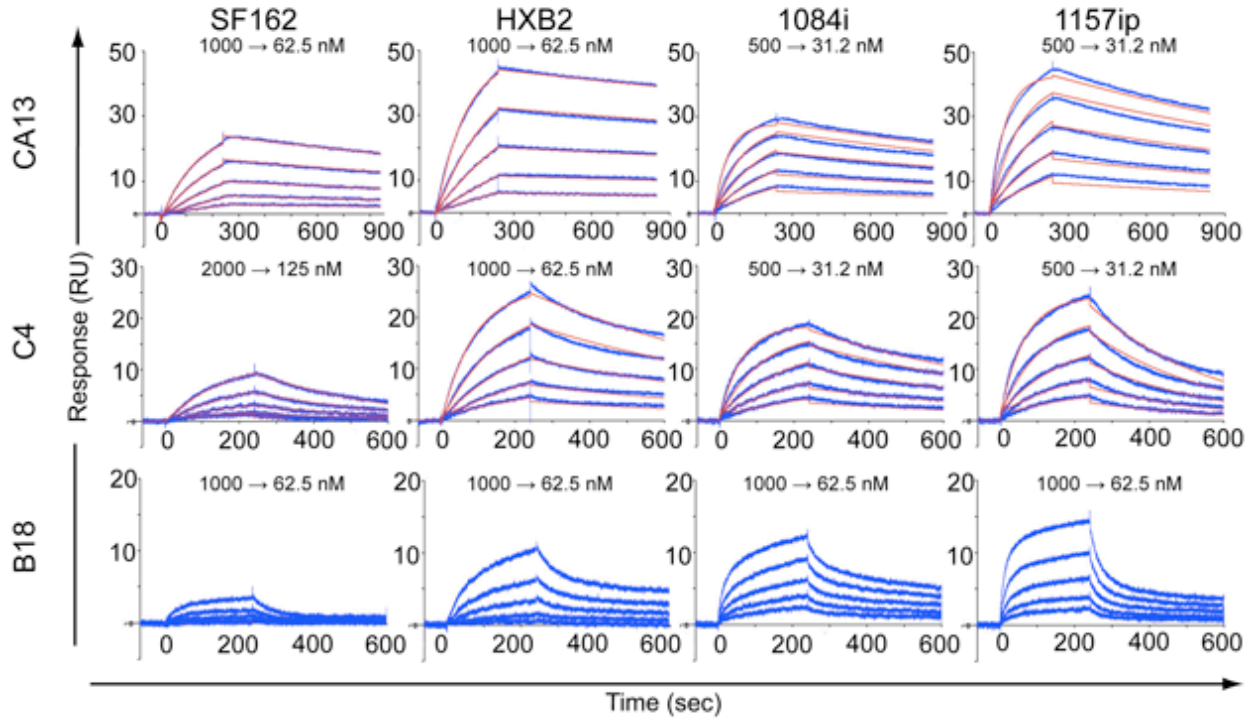

Figure S20. Linear epitope-specific antibody SPR binding curves. Double-reference subtracted SPR binding curves are shown for gp120 binding to captured linear-epitope specific antibodies CA13, C4, or B18 over a series of gp120 concentrations (indicated above each graph). Raw data is shown in blue, and best-fit 1:1 binding model curves are in red. Each column shows data for a different gp120 isolate, and each row shows data for a different antibody. Y-axis is SPR response (in RU) and x-axis is time. Note changes in gp120 concentration range and scale for the Y-axis for each antibody.

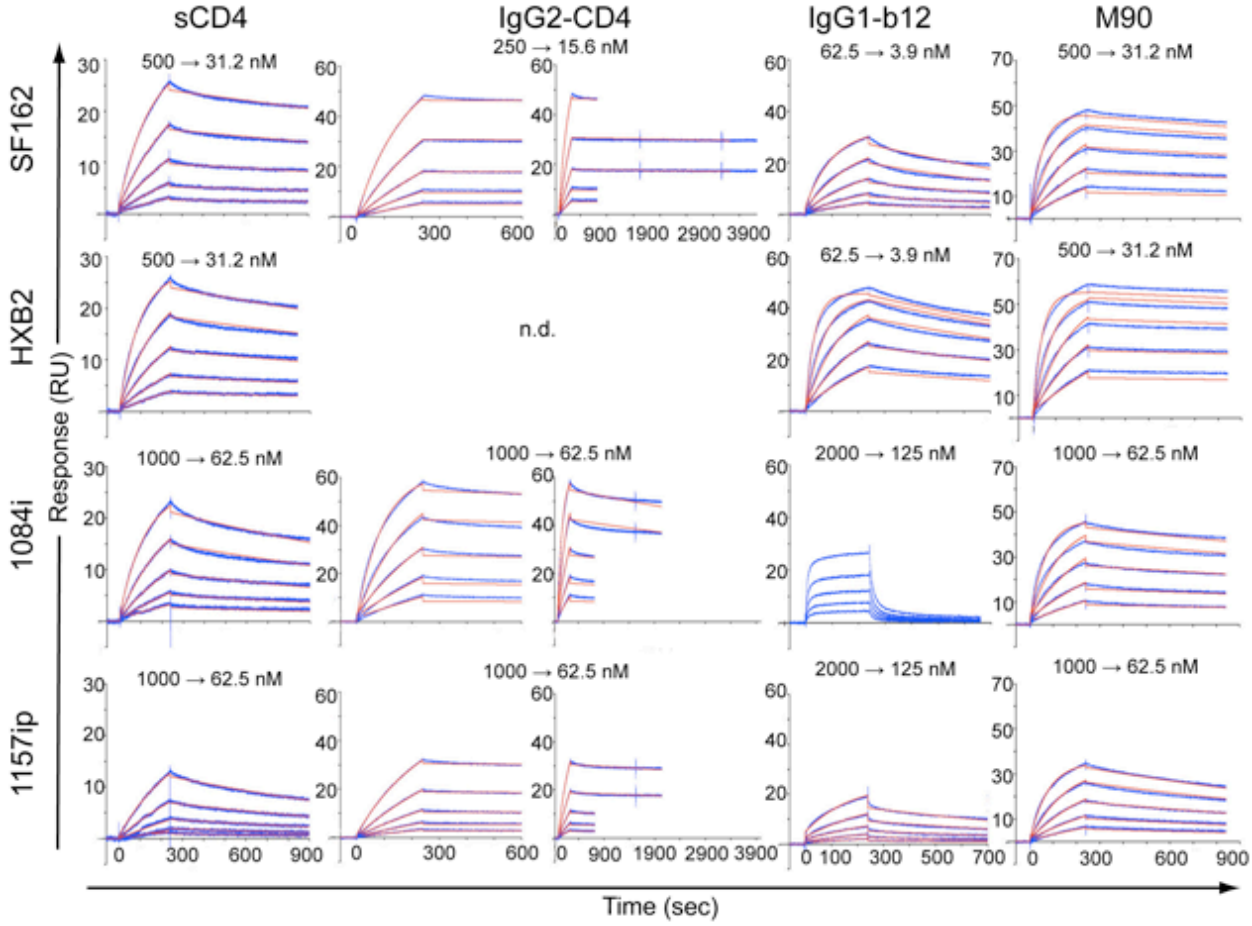

Figure S21. Conformation-dependent antibody SPR binding curves. Double-reference subtracted SPR binding curves are shown for gp120 binding to immobilized (sCD4) or captured conformation-dependent ligands CD4-IgG2, IgG1-b12, and M90 over a series of gp120 concentrations (indicated above each graph, note changes in concentration range among the isolates). Raw data is shown in blue, and best-fit 1:1 binding model curves are in red. Each column shows data for a different gp120 ligand, and each row shows data for a different isolate. Y-axis is SPR response (in RU) and x-axis is time. Note changes in scale for the Y-axis for each ligand. Because IgG2-CD4 dissociation was relatively slow, a longer dissociation phase was used. The early time scale (0-600 sec) is shown on the left, and the full time scale is shown on the right (0-3900 sec).

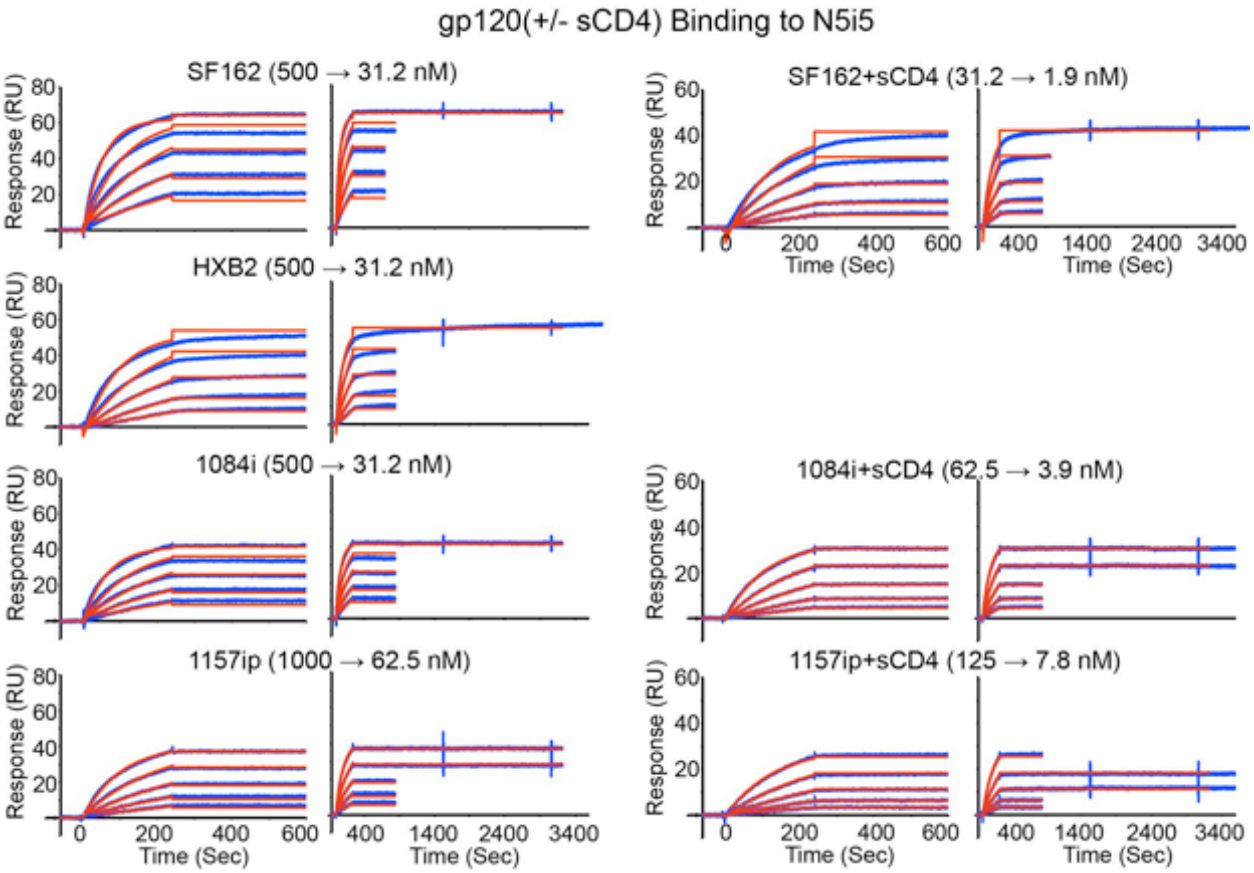

Figure S22. N5i5 SPR binding curves +/- sCD4. Double-reference subtracted SPR binding curves are shown for gp120 (in the presence or absence of 625 nM sCD4) binding to immobilized N5i5 over a series of gp120 concentrations (indicated above each graph). Raw data is shown in blue, and best-fit curves for a 1:1 binding model with the dissociation rate fixed at 0 s^{-1} are in red. Each row shows data for a different gp120 isolate, and each column shows data for that isolate in the absence (left) or presence (right) of sCD4. Y-axis is SPR response (in RU) and x-axis is time. Note changes in scale for the Y-axis, and differences in gp120 concentration series used in the experiment. Because N5i5 dissociation was slow, a longer dissociation phase was used. Within each column, the early time scale (0-600 sec) is shown on the left, and the full time scale is shown on the right (0-3900 sec).

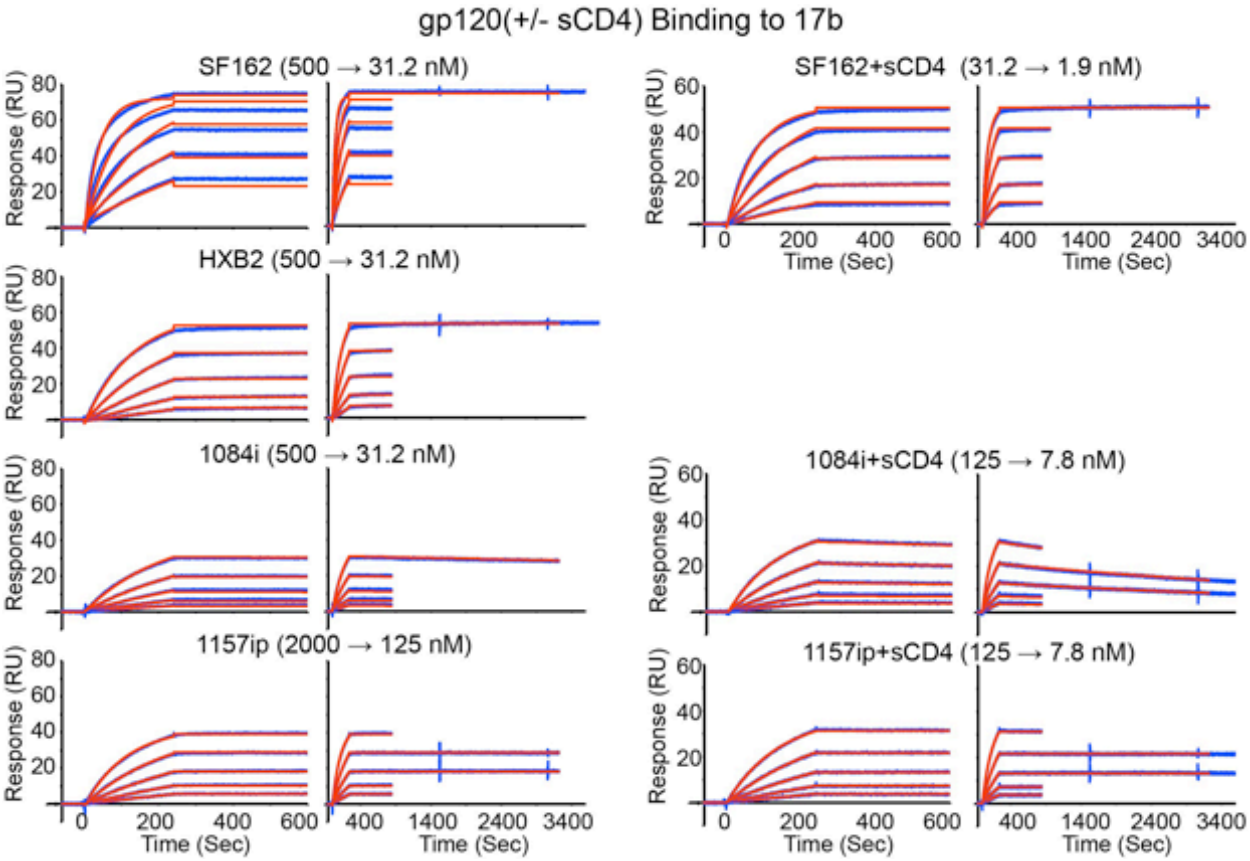

Figure S23. 17b SPR binding curves +/- sCD4. Double-reference subtracted SPR binding curves are shown for gp120 (in the presence or absence of 625 nM sCD4) binding to immobilized 17b over a series of gp120 concentrations (indicated above each graph). Raw data is shown in blue, and best-fit curves for a 1:1 binding model with the dissociation rate fixed at 0 s^{-1} are in red. In the case of 1084i, there was sufficient dissociation to fit the dissociation rate as well using a standard 1:1 binding model. Each row shows data for a different gp120 isolate, and each column shows data for that isolate in the absence (left) or presence (right) of sCD4. Y-axis is SPR response (in RU) and x-axis is time. Note changes in scale for the Y-axis and gp120 concentration series used in the experiment. Because 17b dissociation was slow, a longer dissociation phase was used. Within each column, the early time scale (0-600 sec) is shown on the left, and the full time scale is shown on the right (0-3900 sec).

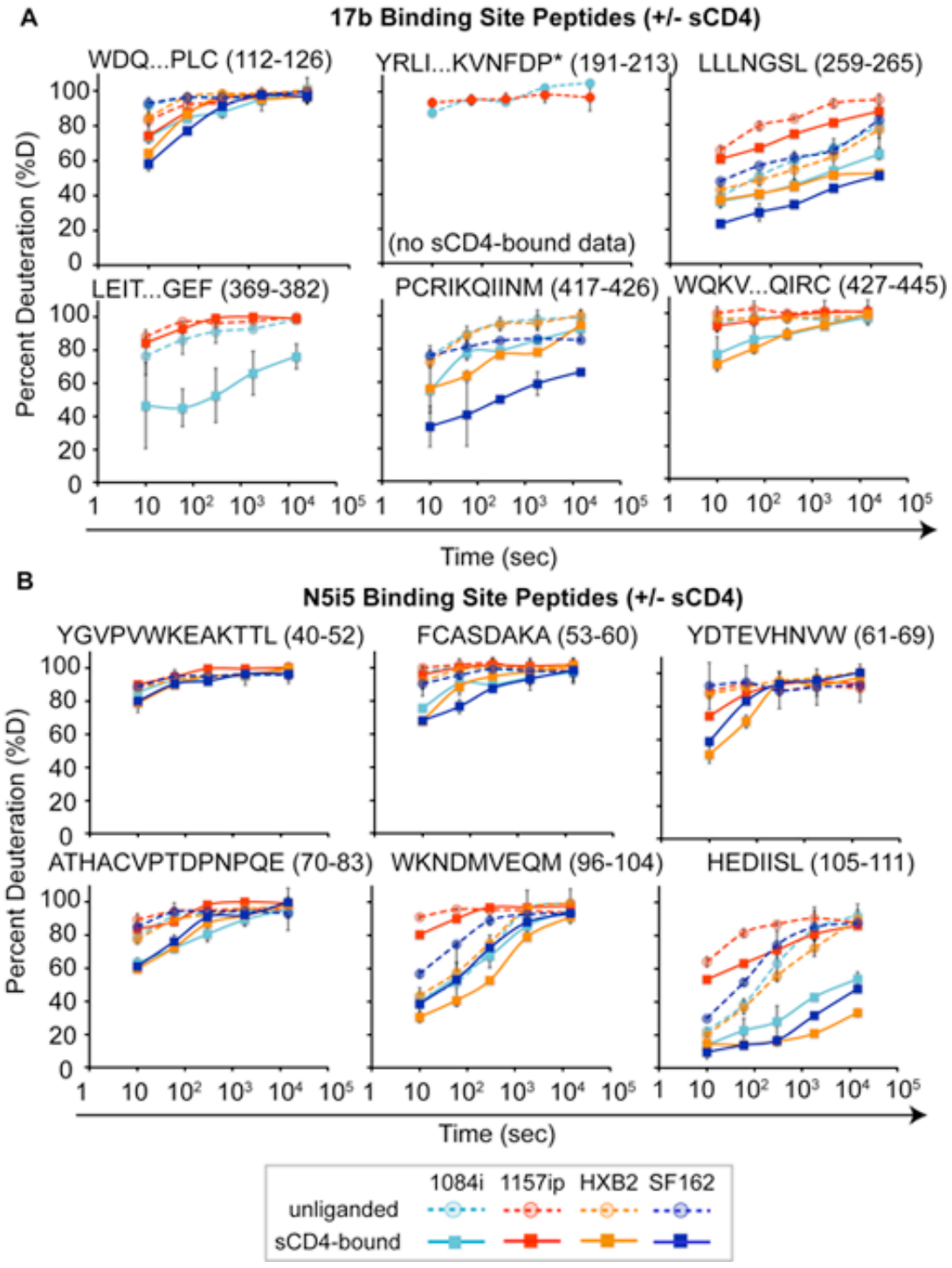

Figure S24. sCD4-induced stabilization of peptides within 17b and N5i5 epitopes. Percent deuteration plots for peptides containing CD4-induced antibody contact residues are shown for peptides in the 17b (A) and N5i5 (B) epitopes in the presence and absence of sCD4. Dashed lines with circles indicate data for unliganded gp120, solid lines with squares indicate data for sCD4-bound gp120. Each isolate is represented by a different color as indicated in the figure legend. It can be seen that CD4i antibody epitopes are differentially stabilized in the different gp120 isolates as a result of sCD4 binding.

Table S1. Summary of SPR Experimental Details

Ligand	Immobilization	Density (RU)	Analyte	Flow rate (ul/min)	Concentration Series (nM)	On (sec)	Off 1 (sec)	Off 2 (sec)	Off 2 Concs (nM)	Fitted Concs
sCD4	EDC/NHS	85	HXB2	40	1000 -> 7.8	240	600	n/a	n/a	500 -> 31.2
		85	SF162	40	1000 -> 15.6	240	780	n/a	n/a	500 -> 31.2
		185	1084i	40	1000 -> 15.6	240	660	n/a	n/a	1000 -> 62
		185	1157ip	40	1000 -> 15.6	240	780	n/a	n/a	1000 -> 62
IgG2-CD4	G α H Fc cap*	80	HXB2		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		80	SF162	40	250 -> 3.9	240	720	3600	125, 62.5	250 -> 15.6
		80	1084i	40	2000 -> 31.2	240	720	1800	1000, 500	1000 -> 62
		80	1157ip	40	2000 -> 31.2	240	720	1800	1000, 500	1000 -> 62
IgG1-b12	G α H Fc cap	50	HXB2	50	500 -> 3.9	240	480	n/a	n/a	62 -> 3.9
		75	SF162	40	250 -> 3.9	240	720	3600	125, 62.5	62 -> 3.9
		75	1084i	40	2000 -> 31.2	240	720	1800	1000, 500	not 1:1
		75	1157ip	40	2000 -> 31.2	240	720	1800	1000, 500	2000 -> 125
17b	G α H Fc cap	60	HXB2	40	1000 -> 31.2	240	600	4200	1000, 500	500 -> 31.2*
		60	SF162	40	1000 -> 31.2	240	600	4200	1000, 500	500 -> 31.2*
		60	1084i	40	2000 -> 31.2	240	600	3600	1000, 500	1000 -> 31.2
		60	1157ip	40	2000 -> 31.2	240	600	3600	1000, 500	2000 -> 125*
17b	G α H Fc cap	50	HXB2 + sCD4	40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		50	SF162 + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	31.2 -> 1.9*
		50	1084i + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	125 -> 7.8
		50	1157ip + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	125 -> 7.8*
N5i5	G α H Fc cap	60	HXB2	40	1000 -> 31.2	240	600	4200	1000, 500	500 -> 31.2*
		60	SF162	40	1000 -> 31.2	240	600	4200	1000, 500	500 -> 31.2*
		60	1084i	40	2000 -> 31.2	240	600	3600	1000, 500	500 -> 31.2*
		60	1157ip	40	2000 -> 31.2	240	600	3600	1000, 500	1000 -> 62.5*
N5i5	G α H Fc cap	50	HXB2 +sCD4	40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		50	SF162 + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	31.2 -> 1.9*
		50	1084i + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	62.5 -> 3.9*
		50	1157ip + sCD4	40	125 -> 1.9	240	720	4500	62.5, 31.2	125 -> 7.8*
M90	G α M Fc cap**	50	HXB2	40	2000 -> 31.2	240	660	n/a	n/a	500 -> 31.2
		50	SF162	40	2000 -> 31.2	240	660	n/a	n/a	500 -> 31.2
		50	1084i	40	2000 -> 31.2	240	660	n/a	n/a	1000 -> 62.5
		50	1157ip	40	2000 -> 31.2	240	660	n/a	n/a	1000 -> 62.5
B18	G α M Fc cap	75	HXB2	40	2000 -> 31.2	240	900	n/a	n/a	not 1:1
		75	SF162	40	1000 -> 31.2	240	720	n/a	n/a	not 1:1
		75	1084i	40	2000 -> 31.2	240	900	n/a	n/a	not 1:1
		75	1157ip	40	2000 -> 31.2	240	900	n/a	n/a	not 1:1
C4	G α M Fc cap	75	HXB2	40	2000 -> 31.2	240	900	n/a	n/a	1000 -> 62.5
		75	SF162	40	1000 -> 31.2	240	720	n/a	n/a	1000 -> 62.5
		75	1084i	40	2000 -> 31.2	240	900	n/a	n/a	500 -> 31.2
		75	1157ip	40	2000 -> 31.2	240	900	n/a	n/a	500 -> 31.2
CA13	G α M Fc cap	70	HXB2	40	2000 -> 31.2	240	900	n/a	n/a	2000 -> 125
		70	SF162	40	2000 -> 31.2	240	720	n/a	n/a	1000 -> 62.5
		70	1084i	40	4000 -> 31.2	240	900	n/a	n/a	1000 -> 62.5
		70	1157ip	40	4000 -> 31.2	240	900	n/a	n/a	1000 -> 62.5

* Goat anti-human, Fc-specific antibody capture
** Goat anti-mouse, Fc-specific antibody capture
n.d. experiment not done

Table S2. Chi^2 error of 1:1 binding vs. fixed dissociation rate (kd = 0 s^{-1}) fits to CD4i antibody SPR data

		Float kd		Fixed kd = 0 s^{-1}	
	Isolate	ka (1/M-s)	Chi2	ka (1/M-s)	Chi2
17b	HXB2	1.52(4) ×10^4*	0.405(7)	1.51(3) ×10^4	0.36(6)
	SF162	5.06(8) ×10^4	4.4(8)	5.06(7) ×10^4	4.4(8)
	1084i	9.5(1) ×10^3	0.32(9)	-	-
	1157ip	4.43(3) ×10^3	0.114(1)	4.38(3) ×10^3	0.121(2)
17b + sCD4	HXB2	-	-	-	-
	SF162	4.24(5) ×10^5	0.332(2)	4.19(6) ×10^5	0.323(1)
	1084i	5.8(5) ×10^4	0.17(9)	-	-
	1157ip	5.59(9) ×10^4	0.07(2)	5.5(1) ×10^4	0.08(2)
N5i5	HXB2	2.2(1) ×10^4	3.0(3)	2.12(6) ×10^4	3.28(4)
	SF162	3.94(3) ×10^4	4.01(9)	3.94(2) ×10^4	3.98(8)
	1084i	3.11(2) ×10^4	1.5(4)	3.12(4) ×10^4	1.50(3)
	1157ip	9.6(2) ×10^3	0.51(5)	9.7(2) ×10^3	0.50(7)
N5i5 + sCD4	HXB2	-	-	-	-
	SF162	2.8(1) ×10^5	1.2(2)	2.9(2) ×10^5	1.2(2)
	1084i	1.6(1) ×10^5	0.3(3)	1.51(3) ×10^5	0.1(4)
	1157ip	6.1(2) ×10^4	0.21(1)	6.1(2) ×10^4	0.214(9)

(-) experiment was not performed or the fixed kd model was not applied
* error on the last significant figure is presented in parentheses as in Table 2 from the main text.

Supporting Methods for Peptide Competition ELISA

Peptides for the peptide competition ELISA were obtained from the NIH AIDS Reagent Program (Consensus Subtype B Env 15-mer peptide set, #9480). The peptide competition ELISA was carried out exactly as described for the standard ELISA in the main text, with a few modifications. SF162 gp120 was plated at 50 ng/well in PBS at 4^{o}C overnight. All peptides were resuspended in dimethyl sulfoxide (DMSO) just prior to use. During the blocking step, antibodies C4 and B18 at 5 μg/mL (or a 1:40 dilution for the CA13 supernatant) were incubated for 1 hour at room temperature with a 40-fold molar excess of peptide. After washing the plate, the antibody/peptide mixture was diluted in a 4-fold concentration series into antibody dilution buffer containing a constant concentration of peptide (~1.2 μM). Matched volumes of DMSO were added to the "no peptide" control samples. Following incubation of the primary antibody for 1 hour at room temperature, washing, secondary antibody, and development were carried out exactly as described for the standard ELISA.