SUPPLEMENTAL INFORMATION

Bradley et al. “Structural Constraints of Vaccine-Induced Tier-2 Autologous HIV Neutralizing Antibodies Targeting the Receptor Binding Site.” (2015)

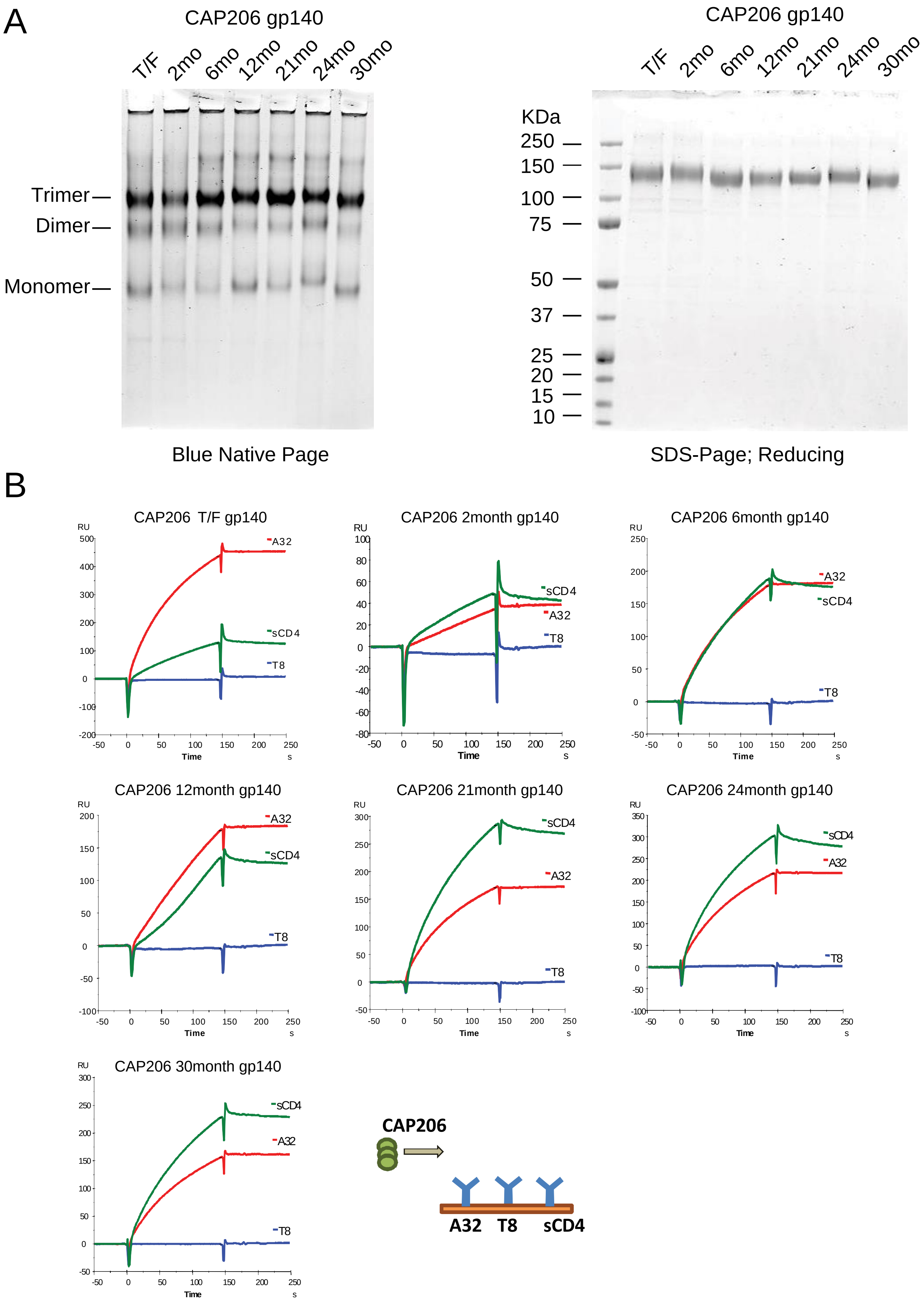

A
CAP206 gp140
T/F
2mo
6mo
12mo
21mo
24mo
30mo
Trimer
Dimer
Monomer
Blue Native Page
CAP206 gp140
KDa
250
150
100
75
50
37
25
20
15
10
SDS-Page; Reducing
B
CAP206 T/F gp140
RU
500
400
300
200
100
0
-100
-200
A32
sCD4
T8
-50
0
50
100
150
200
250
Time
s
CAP206 2month gp140
RU
100
80
60
40
20
0
-20
-40
-60
-80
sCD4
A32
T8
Time
s
CAP206 6month gp140
RU
250
200
150
100
50
0
-50
A32
sCD4
T8
Time
s
CAP206 12month gp140
RU
200
150
100
50
0
-50
-100
A32
sCD4
T8
Time
s
CAP206 21month gp140
RU
300
250
200
150
100
50
0
-50
sCD4
A32
T8
Time
s
CAP206 24month gp140
RU
350
300
250
200
150
100
50
0
-50
-100
sCD4
A32
T8
Time
s
CAP206 30month gp140
RU
300
250
200
150
100
50
0
-50
sCD4
A32
T8
Time
s
CAP206
A32
T8
sCD4

Figure S1. Expression and Purification of CAP206 Env gp140 Proteins, Related to Figure 1. **(A)** Blue-native PAGE and SDS-PAGE of the 7 CAP206 Envs used as immunogens. **(B)** Binding of sCD4, A32 and control mAb T8 to each of the CAP206 Envs by SPR.

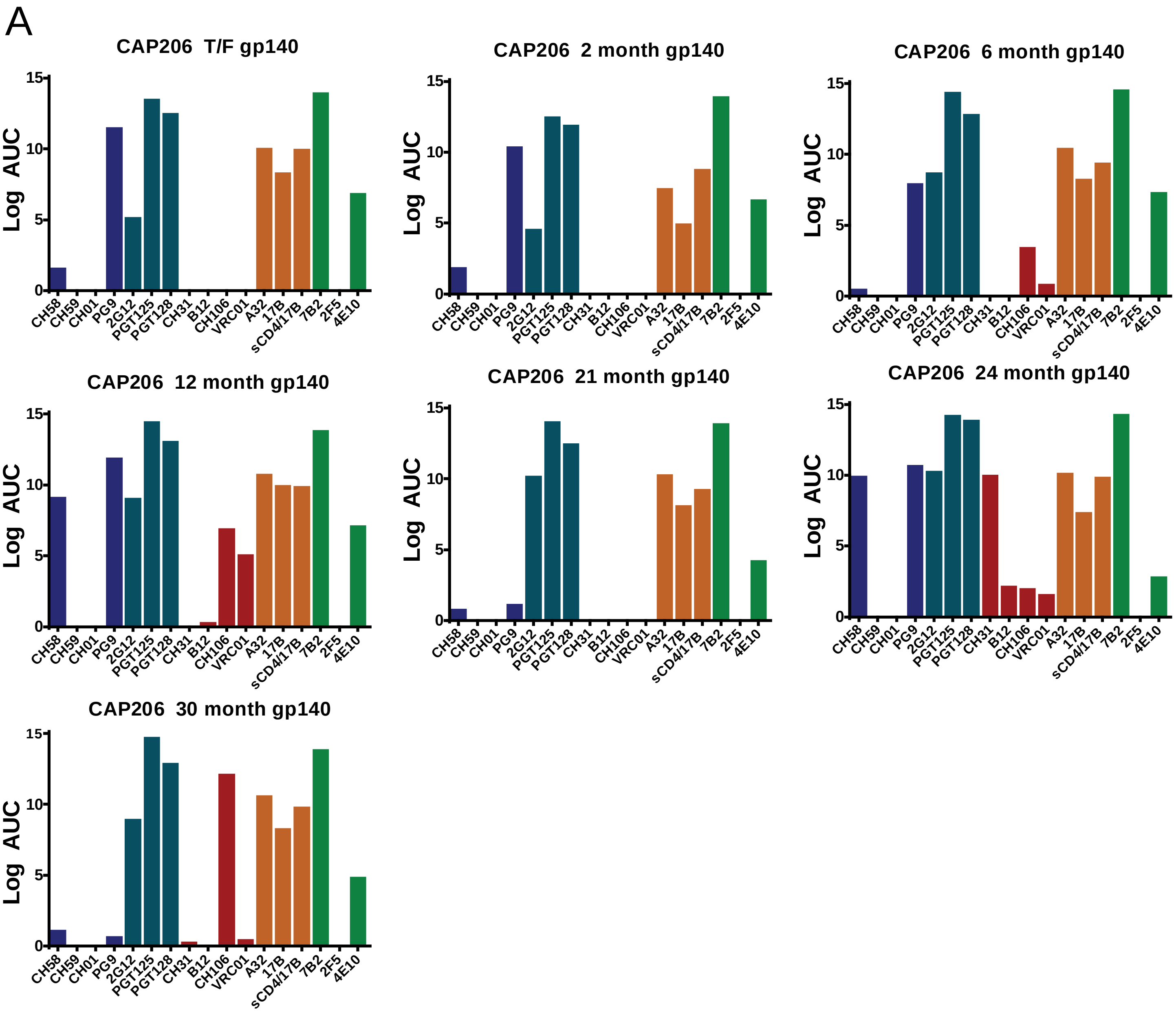
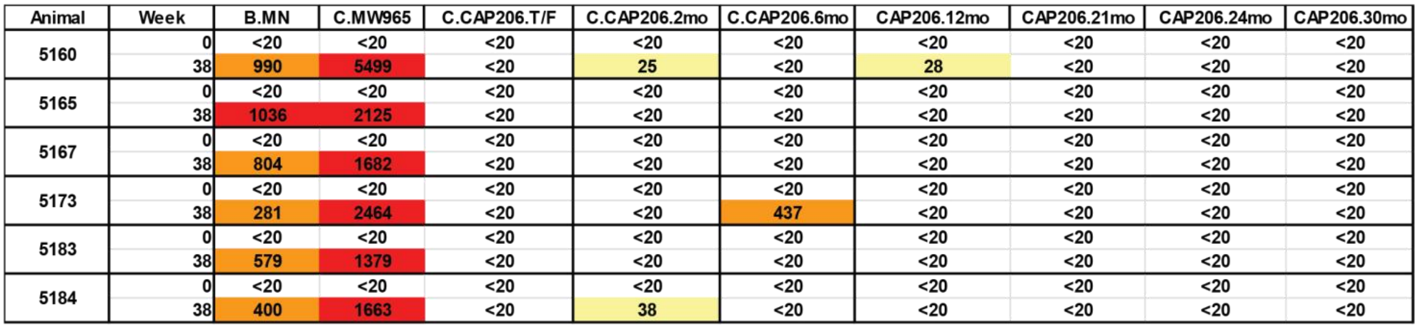


Figure S2. CAP206 Envs can Bind bnAbs Isolated from Infected Individuals, Related to Figure 1. Binding of bnAbs to the 7 CAP206 gp140 immunogens by ELISA (Dark blue, V1/V2; Aqua,V3-glycan; Red,CD4bs; Orange, Other; Green, gp41).

A

Virus ID	IC50 (μg/mL) in TZM-bl cells						
CAP206 Viruses	SA-C2	SA-C8	SA-C62	SA-C72	SA-C74	GMT	Tier
CAP206.TF	647	333	142	549	5000	609	2
CAP206.6mo	254	176	55	265	234	173	2
CAP206.6mo	316	88	264	520	499	286	2
CAP206.12mo	294	304	70	466	589	280	2
CAP206.21mo	584	461	269	1203	5000	847	2
CAP206.24mo	448	287	178	434	555	353	2
CAP206.30mo	667	514	154	765	5000	726	2

B



Animal	Week	B.MN	C.MW965	C.CAP206.T/F	C.CAP206.2mo	C.CAP206.6mo	CAP206.12mo	CAP206.21mo	CAP206.24mo	CAP206.30mo
5160	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
	38	990	5499	<20	25	<20	28	<20	<20	<20
5165	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
	38	1036	2125	<20	<20	<20	<20	<20	<20	<20
5167	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
	38	804	1682	<20	<20	<20	<20	<20	<20	<20
5173	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
	38	281	2464	<20	<20	437	<20	<20	<20	<20
5183	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
	38	579	1379	<20	<20	<20	<20	<20	<20	<20
5184	0	<20	<20	<20	<20	<20	<20	<20	<20	<20
	38	400	1663	<20	38	<20	<20	<20	<20	<20

Figure S3. Plasma Neutralization of CAP206 Env-immunized Animals, Related to Figure 1. (A) Tier phenotype of CAP206 env pseudotyped viruses. CAP206 autologous viruses were tested for neutralization by a characterized panel of sera from clade C HIV infected individuals in the TZM-bl neutralization assay.**(B)**Plasma neutralization of heterologous tier-1 (B.MN, C.MW965) and autologous tier-2 (CAP206 T/F, 2mo, 6mo, 12mo, 21mo,24mo, 30mo) before immunization (week 0) and 2 weeks after the last immunization (week 38) measured as the plasma dilution at which relative luminescence units (RLUs) were reduced 50% compared to control wells in the TZM-bl neutralization assay(Yellow, ID_{50} > 20 Orange, ID_{50} > 200; Red, ID_{50}>1000).

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Heavy
                                             CDR1                    CDR2                                    CDR3
DH427_UCA       EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLEWVSY ISNGGGSTYY ADSVKGRFTI SRDNSKNTLS LQMNSLRAED TAVYYCAKEG WAYFDYWGQG VLVTVSS
DH427_UCA2      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......
DH427_I1        .......... .......... .......... .......... .......... ..IS...... .......... .......... .......... .......... .......... .......
DH427           .......... .......... .......... NS..I..... .......... ..LS.AN... .......... .....Q.... .......V.. ..M....... .S...F.... .......
DH428           .......... .......... .......... T......... .......... ..IS...... T......... .......... ..I....P.. .......... .......... .......

Light
                                         CDR1                CDR2                                            CDR3
DH427_UCA  QAALTQPPSV SKSLGQSVTI SCTGTSNDVG GYNDVSWYQQ HPGTAPRLLI YDVSKRPSGV SDRFSGSKSG NTASLTISGL QAEDEADYYC CSYRSGSTYI FGAGTRLTVL
DH427_UCA2 .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... G......... ..........
DH427_I1   .S........ .......... ......S.I. D..G...... .S........ .......... .......... .......... .......... ....T.G... ..T.......
DH427      .S........ .......... ......S.I. A.TG...... .S........ .......... .......... .......... .TD....... ....T.A... ..T...V...
DH428      .S........ .......... .....NS.I. D..G...... .S........ .......... .......... .......... .......... ....T.G... ..T.......
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Figure S4. Amino Acid Alignment of DH427 Antibody Lineage, Related to Figure 3.

Virus	DH427	DH428
CAP206 T/F	>50	>50
CAP206 2mo	>50	>50
CAP206 6mo	0.02	0.02
CAP206 12mo	>50	>50
CAP206 21mo	>50	>50
CAP206 24mo	>50	>50
CAP206 30mo	>50	>50
B.MN	>50	>50
C.MW965	>50	>50
C.1086	>50	>50
TV1	>50	>50
Q842	>50	>50
Q23	>50	>50
Du172	>50	>50
Du422	>50	>50

Figure S5. DH427 and DH428 Neutralization of Autologous and Heterologous Viruses in the TZM-bl Assay, Related to Figure 4. Values are IC50 of neutralization**.**

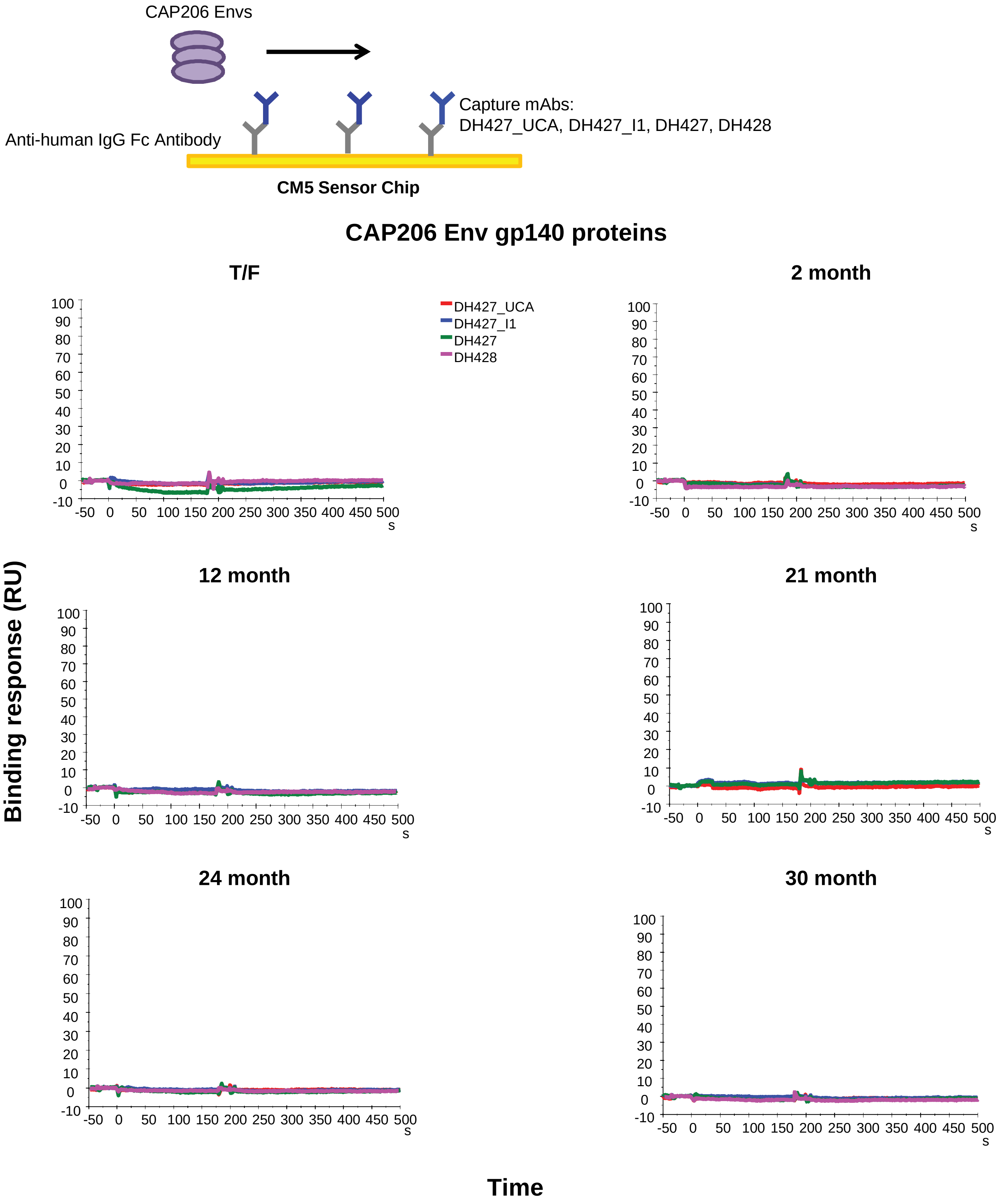
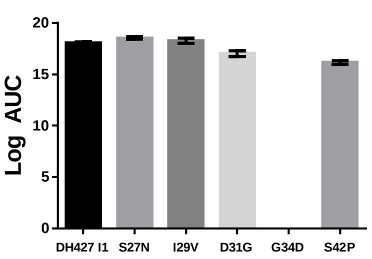
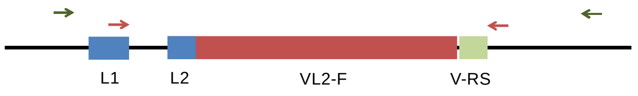


Figure S6. Binding of DH427, DH428 and the Intermediate (I1) and Unmutated Common Ancestor (UCA) to the CAP206 Immunogens by SPR, Related to Figure 5.

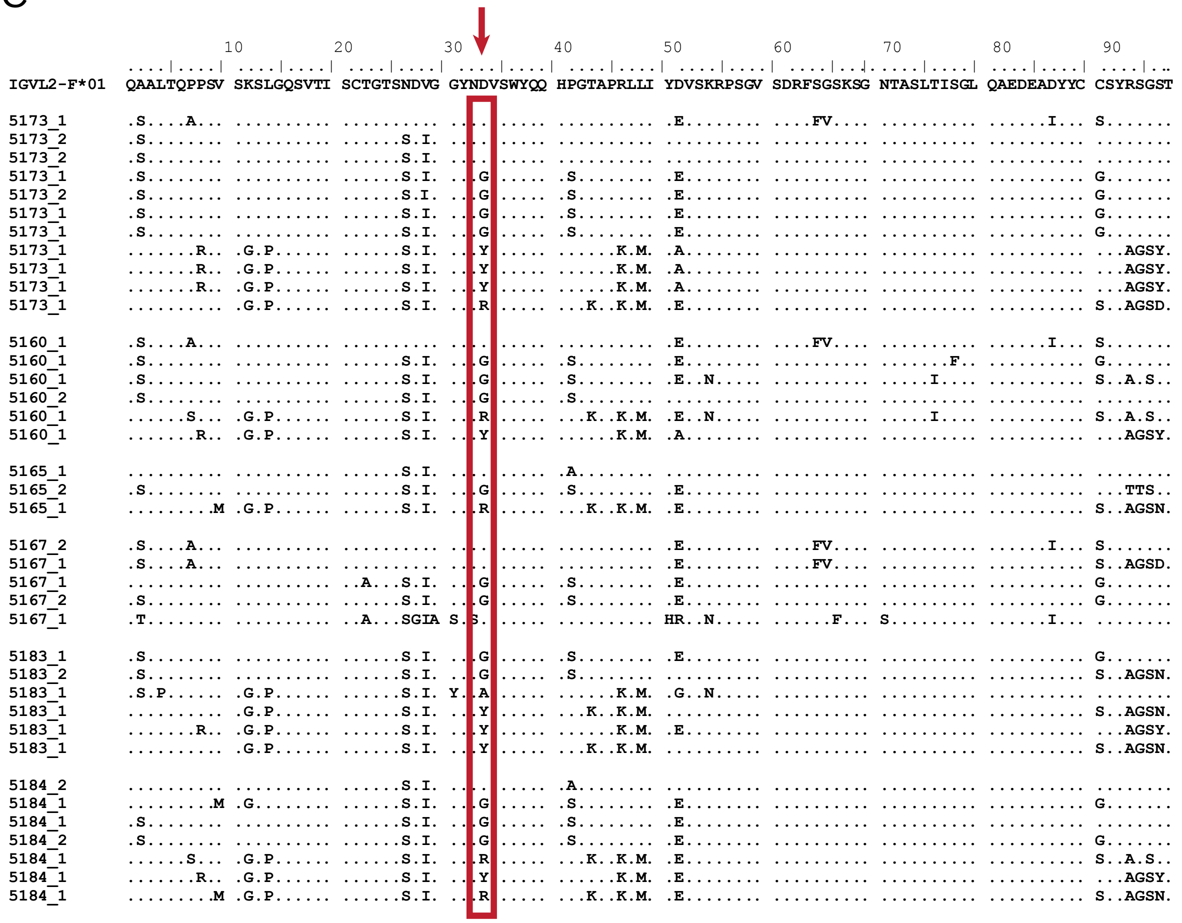
A



B



C



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                       10         20         30         40         50         60         70         80         90
             ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|.... ....|..
IGVL2-F*01   QAALTQPPSV SKSLGQSVTI SCTGTSNDVG GYNDVSWYQQ HPGTAPRLLI YDVSKRPSGV SDRFSGSKSG NTASLTISGL QAEDEADYYC CSYRSGST

5173_1       .S....A... .......... .......... .......... .......... .E........ ....FV.... .......... ......I... S.......
5173_2       .S........ .......... ......S.I. .......... .......... .......... .......... .......... .......... ........
5173_2       .S........ .......... ......S.I. .......... .......... .......... .......... .......... .......... ........
5173_1       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... G.......
5173_2       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... G.......
5173_1       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... G.......
5173_1       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... G.......
5173_1       .......R.. .G.P...... ......S.I. ...Y...... ......K.M. .A........ .......... .......... .......... ...AGSY.
5173_1       .......R.. .G.P...... ......S.I. ...Y...... ......K.M. .A........ .......... .......... .......... ...AGSY.
5173_1       .......R.. .G.P...... ......S.I. ...Y...... ......K.M. .A........ .......... .......... .......... ...AGSY.
5173_1       .......... .G.P...... ......S.I. ...R...... ...K..K.M. .E........ .......... .......... .......... S..AGSD.

5160_1       .S....A... .......... .......... .......... .......... .E........ ....FV.... .......... ......I... S.......
5160_1       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......F.. .......... G.......
5160_1       .S........ .......... ......S.I. ...G...... .S........ .E..N..... .......... .....I.... .......... S..A.S..
5160_2       .S........ .......... ......S.I. ...G...... .S........ .......... .......... .......... .......... ........
5160_1       ......S... .G.P...... ......S.I. ...R...... ...K..K.M. .E..N..... .......... .....I.... .......... S..A.S..
5160_1       .......R.. .G.P...... ......S.I. ...Y...... ......K.M. .A........ .......... .......... .......... ...AGSY.

5165_1       .......... .......... ......S.I. .......... .A........ .......... .......... .......... .......... ........
5165_2       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... ...TTS..
5165_1       .........M .G.P...... ......S.I. ...R...... ...K..K.M. .E........ .......... .......... .......... S..AGSN.

5167_2       .S....A... .......... .......... .......... .......... .E........ ....FV.... .......... ......I... S.......
5167_1       .S....A... .......... .......... .......... .......... .E........ ....FV.... .......... .......... S..AGSD.
5167_1       .......... .......... ..A...S.I. ...G...... .S........ .E........ .......... .......... .......... G.......
5167_2       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... G.......
5167_1       .T........ .......... ..A...SGIA S.S....... .......... HR..N..... ......F... S......... ......I... ........

5183_1       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... G.......
5183_2       .S........ .......... ......S.I. ...G...... .S........ .......... .......... .......... .......... S..AGSN.
5183_1       .S.P...... .G.P...... ......S.I. Y..A...... ......K.M. .G..N..... .......... .......... .......... ........
5183_1       .......... .G.P...... ......S.I. ...Y...... ...K..K.M. .......... .......... .......... .......... S..AGSN.
5183_1       .......R.. .G.P...... ......S.I. ...Y...... ......K.M. .E........ .......... .......... .......... ...AGSY.
5183_1       .......... .G.P...... ......S.I. ...Y...... ...K..K.M. .......... .......... .......... .......... S..AGSN.

5184_2       .......... .......... ......S.I. .......... .A........ .......... .......... .......... .......... ........
5184_1       .........M .G........ ......S.I. ...G...... .S........ .E........ .......... .......... .......... G.......
5184_1       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... ........
5184_2       .S........ .......... ......S.I. ...G...... .S........ .E........ .......... .......... .......... G.......
5184_1       ......S... .G.P...... ......S.I. ...R...... ...K..K.M. .E........ .......... .......... .......... S..A.S..
5184_1       .......R.. .G.P...... ......S.I. ...Y...... ......K.M. .E........ .......... .......... .......... ...AGSY.
5184_1       .........M .G.P...... ......S.I. ...R...... ...K..K.M. .E........ .......... .......... .......... S..AGSN.
```

D

Animal	Week	SSA	SSB	Sm	RNP	Scl 70	Jo 1	dsDNA	Cent B	Histone
5160	0	3	9	6	5	5	4	18	9	7
5165	0	5	6	12	16	28	8	51	14	19
5167	0	3	4	6	11	8	8	27	5	9
5173	0	9	18	5	25	24	13	33	7	12
5183	0	3	6	10	20	7	5	45	12	15
5184	0	8	6	8	7	46	10	33	10	12
5160	38	3	4	7	11	4	10	28	7	10
5165	38	5	7	6	20	12	6	37	9	12
5167	38	2	7	5	6	13	4	15	3	4
5173	38	7	10	8	48	20	22	28	12	13
5183	38	7	9	7	7	8	6	47	5	11
5184	38	5	7	8	5	20	10	24	3	6
4E10	50µg/ml	122	190	10	13	5	186	5	7	18
Synagis	50µg/ml	4	6	4	2	2	6	2	2	2

E

Antibody (50µg/ml)	SSA	SSB	Sm	RNP	Scl 70	Jo 1	dsDNA	Cent B	Histone
DH427 UCA2	7	18	4	4	5	13	14	47	15
DH427 I1	3	4	2	2	2	3	12	2	4
DH427	3	3	3	2	2	2	8	3	2
DH428	4	5	3	5	4	4	19	7	7
4E10	100	192	5	31	11	210	0	19	29
Synagis	5	10	3	2	3	8	8	10	5

Figure S7. Sequencing Genomic DNA to Identify Genomic V_L Protein Sequences, Related to Figure 5 (A) Binding of DH427 I1 and DH427 I1 with light chain mutations to the CAP206 6 month Env measured by ELISA. **(B**) the primer design for determination of IGVL2-F sequences are shown with leader sequences (blue), coding sequence (red), V recombination site (V-RS; green) and noncoding genomic regions (black). 2 primer sets were used to ensure amplification of unrearranged germ-line DNA (green and red arrows). **(C)** Amino acid sequence alignments of germ-line IGVL2-F alleles from all 6 CAP206 animals compared with the annotated IGVL20F allele. 1 indicates sequences amplified with primer set 1 (green) and 2 indicated sequences amplified with primer set 2 (red). Red box highlights residue critical for DH427 binding to the CAP206 Env. **(D-E)** Plasma (**D**) and DH427 antibody lineage (**E**) screened for reactivity with auto-antigens in the AtheNA ANA assay. Values greater than 100 considered positive (orange), and 4E10 (auto-reactive HIV mAb) and Synagis (RSV-specific mAb) used as positive and negative control, respectively.

Table S1. DH427UCA2 Reactive Host Proteins, Related to Figure 5.

Description	DH427UCA2 MFI	Control MFI
family with sequence similarity 21, member C (FAM21C)	6195	-6
family with sequence similarity 21, member C (FAM21C)	6490	-6
G-protein signaling modulator 3 (AGS3-like, C. elegans) (GPSM3)	3085	5
G-protein signaling modulator 3 (AGS3-like, C. elegans) (GPSM3)	2824	44
SNAP25-interacting protein (SNIP)	1057	27
SNAP25-interacting protein (SNIP)	1645	3
nuclear gene encoding mitochondrial protein(ATP5H)	1230	4
nuclear gene encoding mitochondrial protein (ATP5H)	1203	18
WD repeat domain 45 (WDR45), transcript variant 1	452	1
WD repeat domain 45 (WDR45), transcript variant 1	584	16
regulator of G-protein signaling 8 (RGS8), transcript variant 1	2709	87
regulator of G-protein signaling 8 (RGS8), transcript variant 1	2961	90
lactate dehydrogenase A (LDHA)	1086	29
lactate dehydrogenase A (LDHA)	868	10
Microtubule-associated serine/threonine-protein kinase-like	323	89
Microtubule-associated serine/threonine-protein kinase-like	9011	126

Table S2. Data Collection and Refinement Statistics, Related to Figure 6.

	DH427	DH428	DH427/gp120 core
Data collection			
Space group	$P2_12_12_1$	$P2_122_1$	$P6_4$
Cell dimensions			
a,b,c (Å)	78.6, 154.2, 163.6	73.1, 74.6, 102.6	162.9, 162.9, 229.9
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 120
Resolution (Å)	49.02 – 2.69 (2.74 – 2.69)*	74.63 – 2.37 (2.41 – 2.37)	141.08 – 6.63 (6.80-6.63)
R_{sym} or R_{merge}	17.1 (92.2)	8.6 (74.4)	27.3 (233.1)
$I / \sigma I$	7.0 (1.3)	14.6 (2.2)	7.9 (1.1)
Completeness (%)	95.1 (85.5)	92.4 (95.0)	93.7 (94.1)
Redundancy	4.8 (4.7)	4.0 (3.9)	6.7 (6.6)
Refinement			
Resolution (Å)	48.90 - 2.66	60.36 – 2.32	141.08 – 6.63
No. reflections	55460	22892	5756
R_{work} / R_{free} (%)	23.4/26.8	23.9/26.4	34.2/36.5
No. atoms	13104	3232	11602
Protein	12644	3167	11602
Water	460	65	0
B-factors			
Protein	19.9	49.9	2.1
Solvent	17.6	49.4	--
R.m.s. deviations			
Bond lengths (Å)	0.004	0.006	0.013
Bond angles (°)	0.91	1.12	1.81

*Values in parentheses are for highest-resolution shell. One crystal was used for data collection for the DH428 Fab. Multiple crystals were used for DH427 and the complex.