SUPPLEMENTARY MATERIAL

Viral variants that initiate and drive maturation of V1V2-directed HIV-1 broadly neutralizing antibodies

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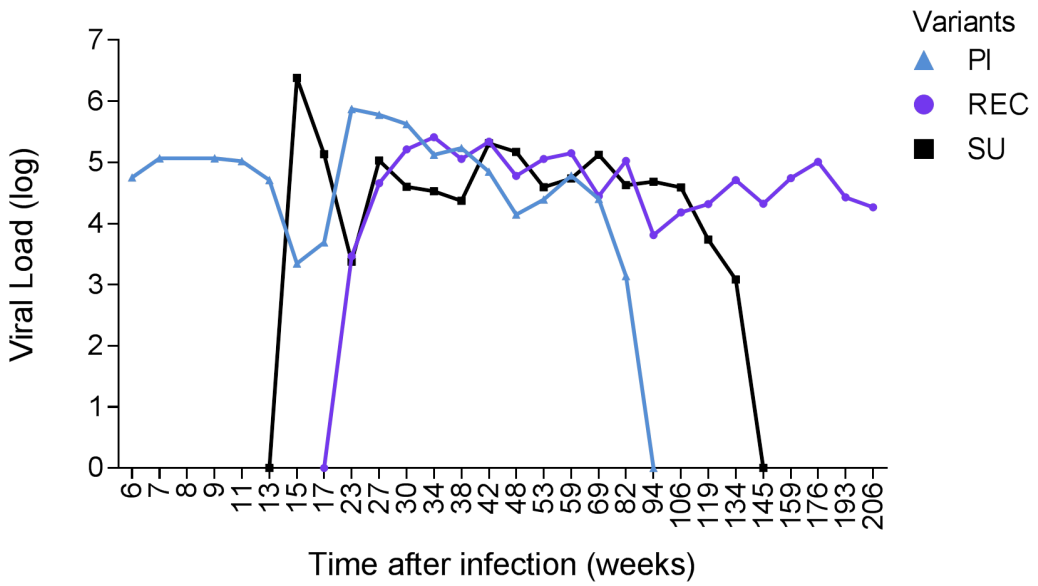
Primer name	Sequence
env_C3_cDNA primer	5'-GCCTTGCCACACGCTCAGNNNNNNNNNGTTGTAAYTTCTAGRTCCCCTCCTG-3'
env_V1V2_F1_1	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTATGGGATSAAAGYCTMAARCCATGTG-3'
env_V1V2_F1_2	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNTATGGGATSAAAGYCTMAARCCATGTG-3'
env_V1V2_F1_3	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNTATGGGATSAAAGYCTMAARCCATGTG-3'
env_V1V2_F1_4	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNTATGGGATSAAAGYCTMAARCCATGTG-3'
Univ_i7_Rev_1	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCTTGCCACACGCTCAG-3'
Univ_i7_Rev_2	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNGCCTTGCCACACGCTCAG-3'
Univ_i7_Rev_3	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNGCCTTGCCACACGCTCAG-3'
Univ_i7_Rev_4	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNGCCTTGCCACACGCTCAG-3'

Supplementary Table 1 PCR primers used to prepare V1V2 amplicons for MiSeq next-generation sequencing.



Supplementary Figure 1 Change in viral load over time in CAP256. Sequences were classified as PI-like (black), SU-like (blue) or REC (purple) based on their relative Hamming distance away from PI, as described in Fig 1a. The number of viral copies for each population was derived from its relative proportion of the total viral load per time point (y-axis) and is shown versus weeks after infection (x-axis).

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a

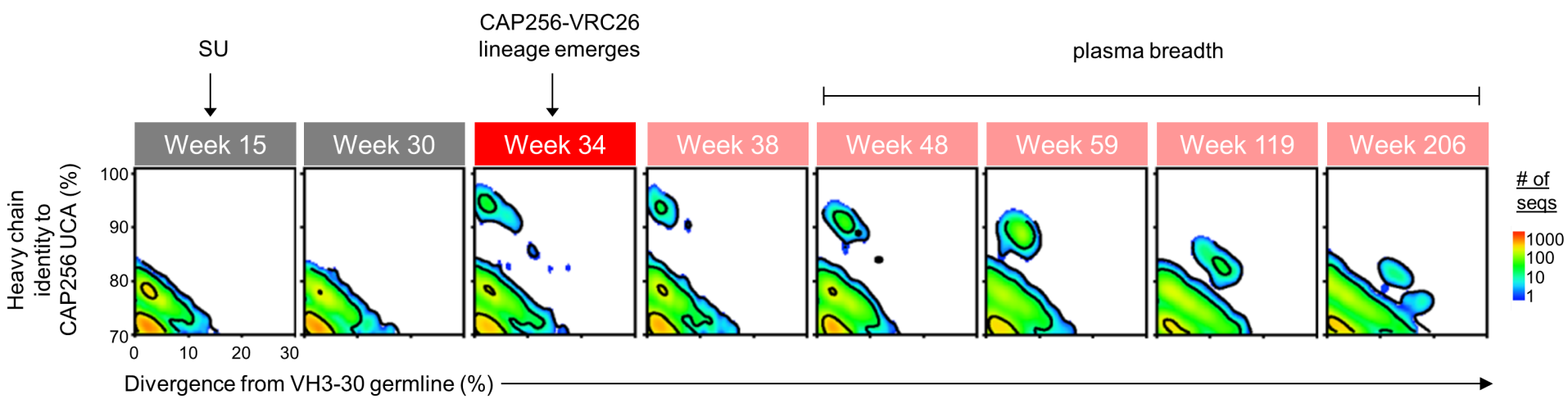
Weeks p.i.	Read count (post quality filter)	Number of consensus sequences	Amino acid frequenices at positon 169 (%)											
			-	A	E	G	H	I	K	L	Q	R	T	M
6	39105	1108	0	0	0	0	0	0	0	0	100	0	0	0
7	92828	1280	0	0	0	0	0	0	0	0	100	0	0	0
8	76182	689	0.1	0	0	0	0	0	0	0.1	99.6	0.1	0	0
9	56436	551	0.4	0	0	0	0	0	0	0	99.5	0.2	0	0
11	59209	563	0	0	0	0	0	0	0	0	100	0	0	0
13	38617	541	0	0	0	0	0	0	0	0	99.8	0.2	0	0
15	139026	3228	0	0	0	0	0	0	99.8	0	0.1	0	0	0
17	21526	405	0	0	2	0	0	0	94	0	3	0	0	0
23	64451	2839	0	0	0	0	0	0	0.2	0	99.6	0	0	0
27	80750	2515	0	0	0.2	0	0	0	13	0	86	0	1	0
30	35354	195	0	0	1	0	0	0	3	0	93	1	3	0
34	92614	937	0	0	0.3	0	0	3.2	3	0	92	0.5	0.4	0
38	96949	815	0	0	0	0	0.1	3	1	0	93	1	2	0
42	62527	906	0	0.4	1	0	0.1	30	1	0	59	4	4	0.1
48	49555	498	0	0	1	0.2	0	47	0	0	52	0	1	0
53	59529	567	0	0	0	0	0	10	0.4	0	89	0	0	0
59	43827	546	0	0	4	0	0	8	0.2	0	88	0	0	0
69	20937	261	0	0	8	0	0	10	4	0.4	78	0	0	0
82	32402	218	0	0	1	0	0	0	0	0	99	0	0	0
94	23847	265	0.4	0	10	0	0	79	3	0	8	0	0	0
106	22058	220	0	0	25	0	0	58	0.5	0	16	0	0	0
119	32228	503	0	0	11	0	0	22	2	0	66	0	0	0
134	16784	262	0	0	35	0	0	2	0.4	0	62	0	0	0
145	30809	421	0	0	79	0	0	0.2	0.2	0	21	0	0	0
159	36680	316	0	0	49	0	0	0	0	0	51	0	0	0
176	30745	305	0	0	16	0	0	0	0	0	84	0	0	0
193	54263	724	0	0	98	0	0	0	0	0	2	0	0	0
206	29435	386	0	0	99.7	0	0	0	0	0	0.3	0	0	0

b

Weeks p.i.	Read count (post quality filter)	Number of consensus sequences	Amino acid frequencies at position 166 (%)					
			-	G	K	R	S	T
6	39105	1108	0	0	0	100	0	0
7	92828	1280	0	0	0	100	0	0
8	76182	689	0.1	0	0	99.9	0	0
9	56436	551	1	0.4	0	99.1	0	0
11	59209	563	0	0	0	100	0	0
13	38617	541	0	0	0	100	0	0
15	139026	3228	0	0	0	100	0	0
17	21526	405	0	0	0	100	0	0
23	64451	2839	0	0	0	100	0	0
27	80750	2515	0	0	0	100	0	0
30	35354	195	0	0	0	100	0	0
34	92614	937	0	0	0.1	99.9	0	0
38	96949	815	0	0.1	0.1	99.8	0	0
42	62527	906	0	0	0.3	99.6	0.1	0
48	49555	498	0	0	0.2	99	0	0.4
53	59529	567	0	0	0.2	98	0	1
59	43827	546	0	0	2	86	0.2	11
69	20937	261	0	0	23	76	0	2
82	32402	218	0	0	35	63	0	2
94	23847	265	0.4	0	89	10	0	0.4
106	22058	220	0	0	52	45	0	4
119	32228	503	0	0	90	9	1	0
134	16784	262	0	0	13	49	37	0
145	30809	421	0	0	1	89	10.2	0
159	36680	316	0	0	0	49	51	0
176	30745	305	0	0	0	18	82	0
193	54263	724	0	0	0	98	2	0
206	29435	386	0	0	0	99.7	0.3	0

50 -100%
10 - 49%
0.01 - 9%
0%

Supplementary Figure 2 Frequency of amino acids or immunotypes (%) at positions (a) 169 and (b) 166 in V1V2 next-generation consensus sequences between 6 and 206 weeks after infection. Read depth after quality filtering is shown for each time-point. Green, blue, or purple shading indicates ranges of amino acid frequencies, as shown in the key.



Supplementary Figure 3 Detection of the CAP256-VRC26 lineage in the periphery. Heat map plots show sequence identity to the UCA (y-axis) versus germline divergence (x-axis) for all VH3-30*18 sequences identified in antibody next-generation sequences (NGS) from 15 to 206 weeks. The time of superinfection (SU), the emergence of the CAP256-VRC26 NGS transcripts and onset of plasma breadth are shown. Frequency of sequences is coloured as indicated by the key. Previously published data7 did not include this 34-week time-point.

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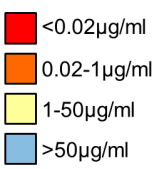
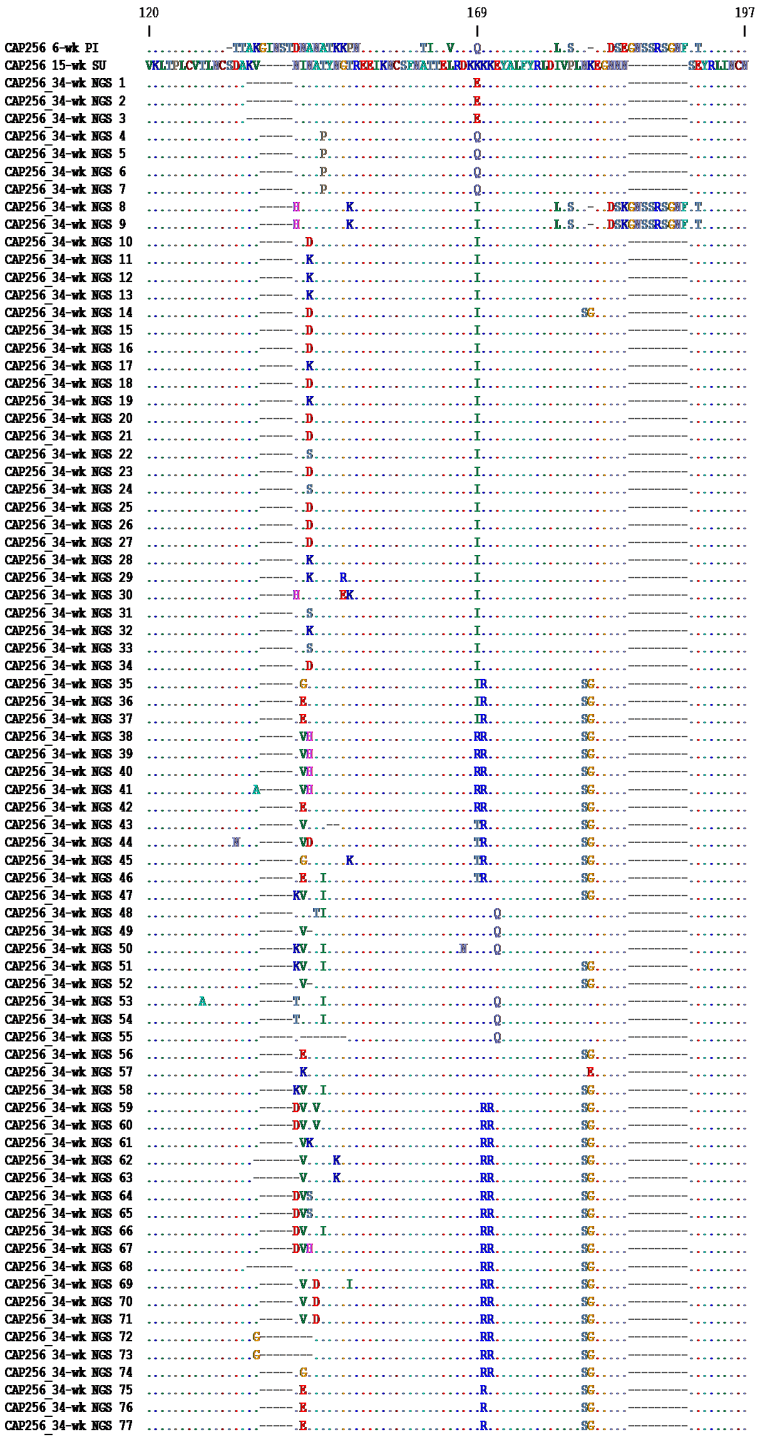
Virus	Clade	IC_{50}
0260.v5.c36	A	>50
0330.v4.c3	A	>50
0439.v5.c1	A	>50
3365.v2.c20	A	>50
3415.v1.c1	A	>50
3718.v3.c11	A	>50
BB201.B42	A	>50
BI369.9A	A	>50
BS208.B1	A	>50
KER2008.12	A	>50
KER2018.11	A	>50
KNH1209.18	A	>50
MB201.A1	A	>50
MB539.2B7	A	>50
MI369.A5	A	>50
MS208.A1	A	>50
Q23.17	A	>50
Q259.17	A	>50
Q769.d22	A	>50
Q769.h5	A	>50
Q842.d12	A	>50
QH209.14M.A2	A	>50
RW020.2	A	>50
UG037.8	A	>50
246-F3.C10.2	AC	>50
3301.V1.C24	AC	>50
3589.V1.C4	AC	>50
6540.v4.c1	AC	>50
6545.V4.C1	AC	>50
0815.V3.C3	ACD	>50
6095.V1.C10	ACD	>50
3468.V1.C12	AD	>50
Q168.a2	AD	>50
Q461.e2	AD	>50
620345.c1	AE	>50
BJOX009000.02.4	AE	>50
BJOX010000.06.2	AE	>50
BJOX025000.01.1	AE	>50
BJOX028000.10.3	AE	>50
C1080.c3	AE	>50
C2101.c1	AE	>50
C4118.09	AE	>50
CNE3	AE	>50
CNE5	AE	>50
CNE55	AE	>50
CNE56	AE	>50
CNE59	AE	>50
CNE8	AE	>50
M02138	AE	>50
R1166.c1	AE	>50
R2184.c4	AE	>50
TH966.8	AE	>50
TH976.17	AE	>50
235-47	AG	>50
242-14	AG	>50
263-8	AG	>50
269-12	AG	>50
271-11	AG	>50
928-28	AG	>50
DJ263.8	AG	>50
T250-4	AG	>50
T251-18	AG	>50
T253-11	AG	>50
T255-34	AG	>50
T257-31	AG	>50
T266-60	AG	>50

Virus	Clade	IC_{50}
T278-50	AG	>50
T280-5	AG	>50
T33-7	AG	>50
3988.25	B	>50
5768.04	B	>50
6101.10	B	>50
6535.3	B	>50
7165.18	B	>50
45_01dG5	B	>50
89.6.DG	B	>50
AC10.29	B	>50
ADA.DG	B	>50
Bal.01	B	>50
BaL.26	B	>50
BG1168.01	B	>50
BL01.DG	B	>50
BR07.DG	B	>50
BX08.16	B	>50
CAAN.A2	B	>50
CNE10	B	>50
CNE12	B	>50
CNE14	B	>50
CNE4	B	>50
CNE57	B	>50
HO86.8	B	>50
HT593.1	B	>50
HXB2.DG	B	>50
JRCSF.JB	B	>50
JRFL.JB	B	>50
MN.3	B	>50
PVO.04	B	>50
QH0515.01	B	>50
QH0692.42	B	>50
REJO.67	B	>50
RHPA.7	B	>50
SC422.8	B	>50
SF162.LS	B	>50
SS1196.01	B	>50
THRO.18	B	>50
TRJO.58	B	>50
TRO.11	B	>50
WITO.33	B	>50
X2278.C2.B1	B	>50
YU2.DG	B	>50
BJOX002000.03.2	BC	>50
CH038.12	BC	>50
CH070.1	BC	>50
CH117.4	BC	>50
CH119.10	BC	>50
CH181.12	BC	>50
CNE15	BC	>50
CNE20	BC	>50
CNE21	BC	>50
CNE40	BC	>50
CNE7	BC	>50
286.36	C	>50
288.38	C	>50
0013095-2.11	C	>50
001428-2.42	C	>50
0077_V1.C16	C	>50
00836-2.5	C	>50
0921.V2.C14	C	>50
16055-2.3	C	>50
16845-2.22	C	>50
16936-2.21	C	>50
25710-2.43	C	>50

Virus	Clade	IC_{50}
25711-2.4	C	>50
25925-2.22	C	>50
26191-2.48	C	>50
3168.V4.C10	C	>50
3637.V5.C3	C	>50
3873.V1.C24	C	>50
6322.V4.C1	C	>50
6471.V1.C16	C	>50
6631.V3.C10	C	>50
6644.V2.C33	C	>50
6785.V5.C14	C	>50
6838.V1.C35	C	>50
96ZM651.02	C	>50
BR025.9	C	>50
CAP210.E8	C	>50
CAP244.D3	C	>50
CAP45.G3	C	>50
Ce1176.A3	C	>50
CE703010217.B6	C	>50
CNE30	C	>50
CNE31	C	>50
CNE53	C	>50
CNE58	C	>50
DU123.06	C	>50
DU151.02	C	>50
DU156.12	C	>50
DU172.17	C	>50
DU422.01	C	>50
MW965.26	C	>50
SO18.18	C	>50
TV1.29	C	>50
TZA125.17	C	>50
TZBD.02	C	>50
ZA012.29	C	>50
ZM106.9	C	>50
ZM109.4	C	>50
ZM135.10a	C	>50
ZM176.66	C	>50
ZM197.7	C	>50
ZM214.15	C	>50
ZM215.8	C	>50
ZM233.6	C	>50
ZM249.1	C	>50
ZM53.12	C	>50
ZM55.28a	C	>50
3326.V4.C3	CD	>50
3337.V2.C6	CD	>50
3817.v2.c59	CD	>50
191821.E6.1	D	>50
231965.c1	D	>50
247-23	D	>50
3016.v5.c45	D	>50
57128.wrc15	D	>50
6405.v4.c34	D	>50
A03349M1.wrc4a	D	>50
NKU3006.ec1	D	>50
UG021.16	D	>50
UG024.2	D	>50
P0402.c2.11	G	>50
P1981.C5.3	G	>50
X1193.c1	G	>50
X1254.c3	G	>50
X1632.S2.B10	G	>50
X2088.c9	G	>50



Supplementary Figure 4 CAP256-VRC26 UCA neutralization of a panel of 196 heterologous HIV-1 viruses from multiple subtypes. Values shown are IC50 values with a starting concentration of 50 µg/ml, and >50 indicating no neutralization, as indicated in the key.



Supplementary Figure 5 Amino acid alignment of the 34-week V1V2 next-generation consensus sequences (HXB2 positions 120-197). Amino acid identity to the superinfecting virus (15-wk SU) is shown as dots, while deletions are shown as dashes. The primary infecting virus (6-wk PI) is also included in the alignment. Residues are coloured according to BioEdit amino acid assignments.

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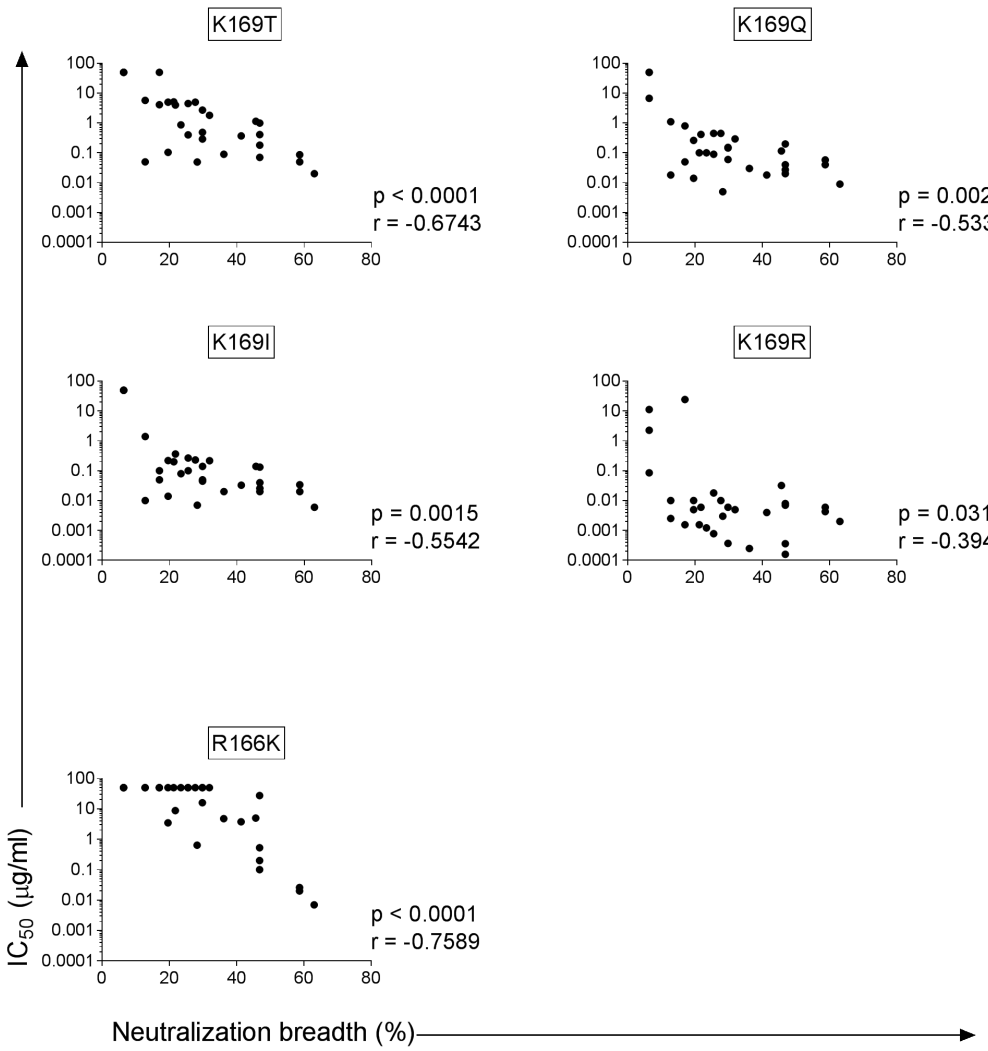

Supplementary Figure 6 CAP256-VRC26 mAb neutralization of 169/166 mutants and correlation with neutralization breadth. A two-tailed Spearman rank test was used to calculate the *r* and *P* values between IC_{50} neutralization titers of K169T/Q/I/R or R166K mutants (IC_{50} titres on the y-axis) and heterologous neutralization breadth (percentage on the x-axis).

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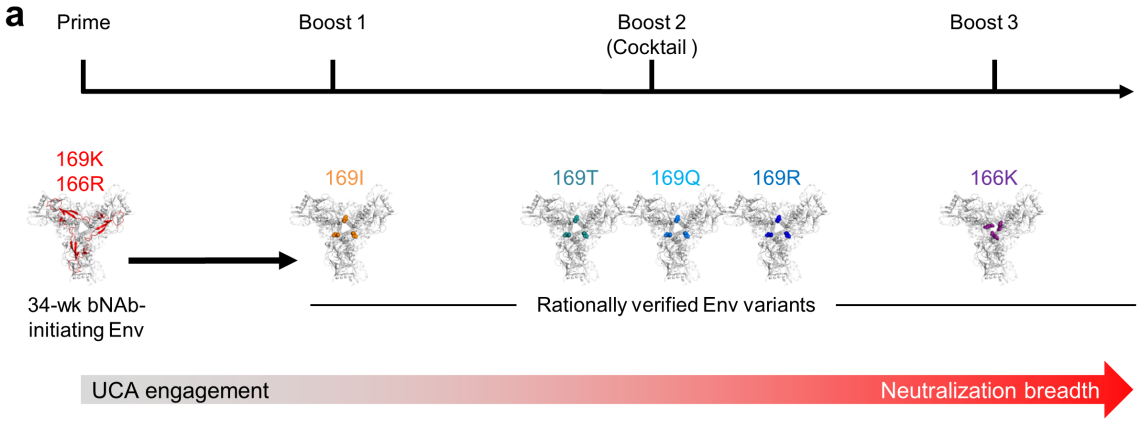

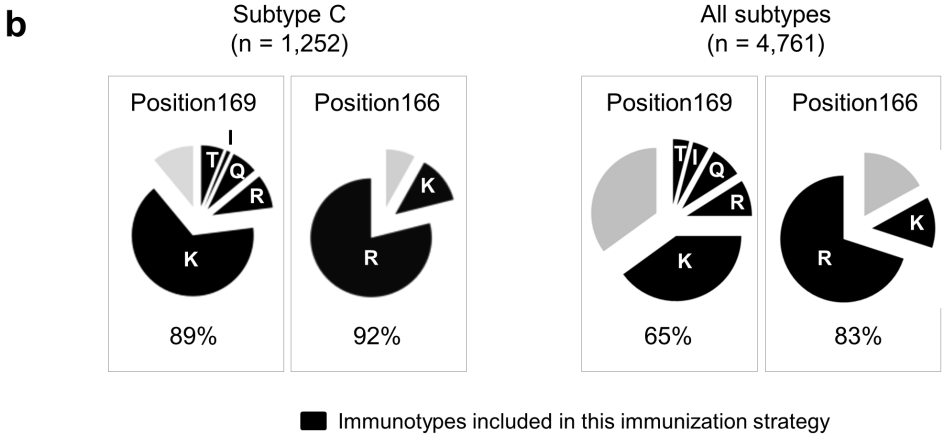

Supplementary Figure 7 Recapitulating viral evolution for vaccine design. (**a)** A proposed prime-sequential boost immunization strategy to elicit V1V2-directed bNAbs, based on the key viral events that initiated and drove neutralization breadth in the CAP256-VRC26 lineage. A CAP256 34-week bNAb-initiating Env (red), which has the 169K and 166R immunotypes, would be used as a prime to engage UCAs with long CDRH3s. This would be followed by three sequential boosts using immunogens with varying immunotypes to mimic the viral diversification in CAP256 that drove bNAb development. The 169I boost (orange) would be administered first, followed by a cocktail of 169T/Q and R immunogens (blue) and a final boost to expose vaccine-induced mAbs to the 166K immunotype (purple). (**b**) Prevalence of major immunotypes among subtype C and all global viruses are shown in pie charts, with those included in the proposed immunization schedule shaded black. The proposed schedule is tailored towards subtype C viruses using experimentally validated immunotypes. However, vaccine coverage may potentially be enhanced for subtype B viruses by addition of a 169V immunotype.

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