Supplemental Online Material

For

An autoreactive antibody from an SLE/HIV-1 individual broadly neutralizes HIV-1

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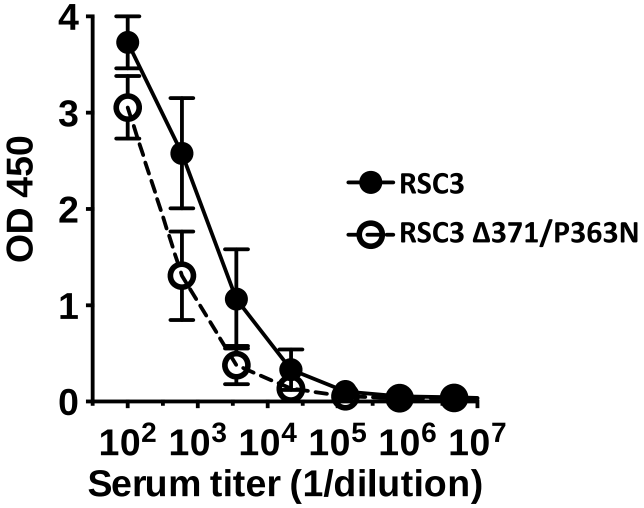
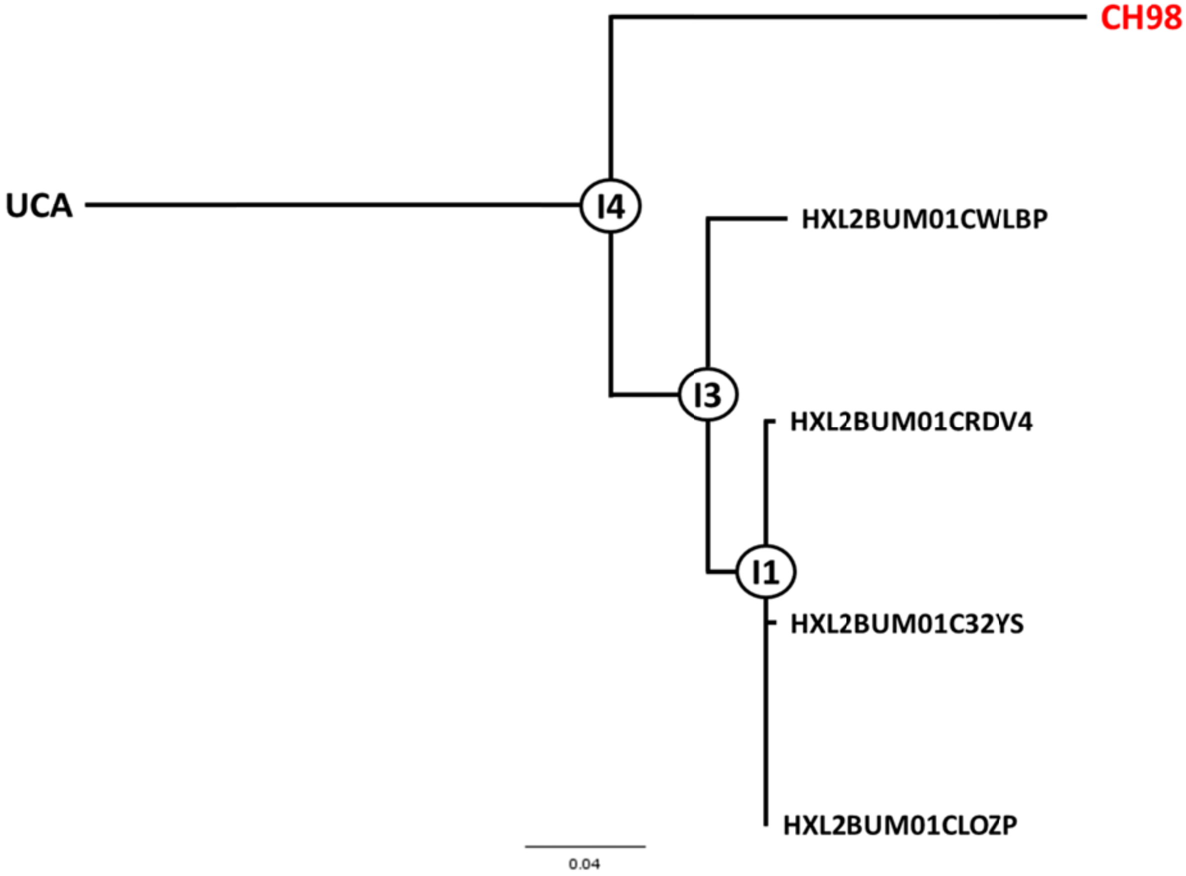


Supplemental Figure 1 - Identification of neutralizing CD4bs antibodies in CH5329 serum. Binding of serial dilutions of CH5329 serum (x-axis) to RSC3 (solid circles) and RSC3Δ371I/P363N (open circles) proteins, expressed as optical density at a 450 nm wavelength (y-axis). Error bars represent standard error of the mean from two independent experiments.

Supplemental Table 1 - Characteristics of the $V_{H/L}$(D)$J_{H/L}$ rearrangement of monoclonal antibody CH98

	$V_{H/L}$	D_H	$J_{H/L}$	CDR3 length[1]	Mutation frequency	Isotype
Heavy chain	3-30	1-IR1	4	21	25.3%	G1
Light chain	λ 2-23		3	12	15.8%	

[1] amino acids



Supplemental Figure 2 - Phylogeny of V_HDJ_H sequences from memory B cells and pyrosequencing. The ancestral reconstruction of the CH98 clonal lineage was performed as described in the Methods. The isolated mature antibody CH98 is shown in red. Pyrosequencing-derived sequences are shown in black. The inferred evolutionary paths to observed matured antibody and pyrosequencing-derived sequences starts with the inferred unmutated common ancestor (UCA) and encompasses three maturationally intermediate V_HDJ_H sequences (I4, I3 and I1, shown in circles).

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                10        20        30        40        50        60        70        80        90
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
UCA     CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGT
I4      ------------------------------------G-A--C----A--------C------------C----T----G---A-A--GAG
CH98    G----T------------------------------G-A--C--A-A--------C-G----G-----C----T----G---A-A-TGAC
I3      -------G------------------------T---G-A--C-A--A--------C------------C----T----G---A-A--GAG
2       -------G------------------------T---G-A--C-A--A--------C------A-----C----T----G---A-A--GAG
I1      ----A--G------------------------T---G-A--C-AC-A--------C------------C----T----G---A-A--GAG
4       ----A--G---------------------T--T---G-A--C-AC-A--------C------------C----T----G---A-A--GAG
3       ----A--G------------------------T---G-A--C-AC-A--------C------------C----T----G---A-A--GAG
1       ----A--G------------------------T---G-A--C-AC-A--------C------------C----T----G---A-A--GAG

               100       110       120       130       140       150       160       170       180
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
UCA     AGCTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAATACTAT
I4      -C-----A---------------------------------T--------------CT--TCT-----CAG------A--CTCG-T-A--
CH98    -CG----A-T-C-----------------------------T--------------CTG-TGT---A-CAG----GAAC-CTCT-T-A-C
I3      -C-----A---------------------------------T--------------CT-CTCT-----CAG------A-GCTCG-T-A--
2       -C-----A---------------------------------T--------------CT-CTCT-----CA-----G-A-G-TCG-T-A--
I1      -C-----A---------------------------------T--------------CT-CTCT-----CAG------A-GCTCG-T-A--
4       -C-----A---------------------------------T--------------CT-CTCT-----CAG------A-GCTCG-T-A--
3       -C-----A---------------------------------T--------------CT-CTCT-----CAG------A-GCTCG-T-A--
1       -C-----A---------------------------------T--------------CT-CTCT-----CAG------A-GCTCG-T-A--

               190       200       210       220       230       240       250       260       270
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
UCA     GCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGAC
I4      ------ATT------------------G-------G----------G-----G-G--A--T----G----G------C--------C---
CH98    ------ATT--C--------C--T-G-G-------G------ATG-G-----AAA--AC-T----G----CG--AG-C-TG-T---C---
I3      T-----ATT-----------------AG----T--G----------G-----G-G--A--T----G---CG------C---C----C---
2       T-----ATT-----------------AG----T--G----------G-----G-G--A--T----G---CG--C---CC--C----C---
I1      T-----ATT-----------------AG----T--G----------GA----G-G--A--T----G---CG------C---C----C---
4       T-----ATT-----------------AG----T--G----------GA----G-G--A--T----G---CG------C---C----C---
3       T-----ATT-----------------AG----T--G----------GA----G-G--A--T----G---CG----T-C---C----C---
1       T-----ATT-----------------AG----T--G----------GA----G-G--A--T----G---CG------C---C----C---

               280       290       300       310       320       330       340       350       360
        ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
UCA     ACGGCTGTGTATTACTGTGCGAAAGAGAGGGGAAGACACAGAACAAGGGACACACTCGAGTTCGGGGAGTTCTTTGACTACTGGGGCCAG
I4      --------C--C-------T---------------------------------------------AA------------C------T---
CH98    ------ACA--C-T-----T---G---------------C-----C----------T--A-----AAGA--T-------CA-----G---
I3      --------C--C-------T---------------------------------------------AA------------C------T---
2       -----C--C----------T--------------C---------G--------------------AA------------C------T---
I1      --------C--C-------T--GG-----------G-----------------------------AA--------C---C------T---
4       --------C--C-------T--GG-----------G-----------------------------AA--------C---C------T---
3       --------C--C-------T--GG-----------G-----------------------------AA--------C---C------T---
1       --------C--C-------T--GG-----------G-----------------------------AA--------C---C------T---

               370       380
        ....|....|....|....|....
UCA     GGAACCCTGGTCACCGTCTCCTCA
I4      ---G---G------------G---
CH98    ---G---G------------G---
I3      ---G---G------A-----G---
2       ---G---G------A-----G---
I1      ---G---G------A-----G---
4       ---G---G------A-----G---
3       ---G---G------A-----G---
1       ---G---G------A-----G---
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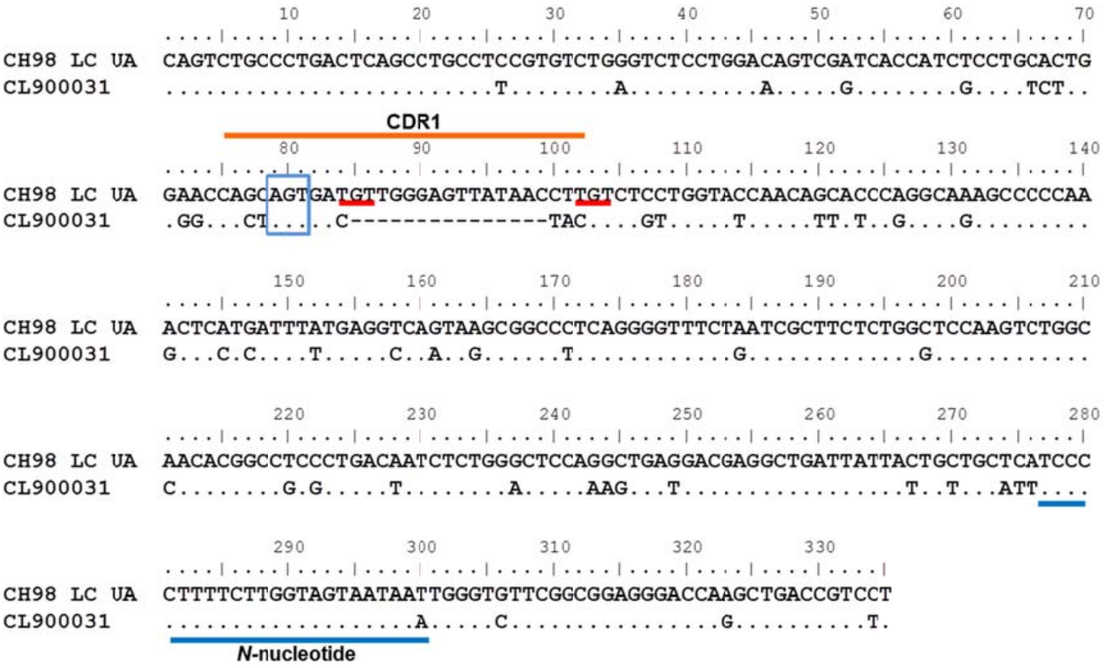
Supplemental Figure 3 - Alignment of the CH98 bNab, 454 pyrosequencing-derived, and inferred V_HDJ_H sequences.



```
                    10        20        30        40        50        60        70
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CH98 LC UA  CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTG
CL900031    .........................T........A..........A.....G........G....TCT..

                    80        90       100       110       120       130       140
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CH98 LC UA  GAACCAGCAGTGATGTTGGGAGTTATAACCTTGTCTCCTGGTACCAACAGCACCCAGGCAAAGCCCCCAA
CL900031    .GG...CT.....C---------------TAC....GT.....T.....TT.T..G....G.........

                   150       160       170       180       190       200       210
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CH98 LC UA  ACTCATGATTTATGAGGTCAGTAAGCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGC
CL900031    G...C.C....T.....C..A..G......T............G.............G............

                   220       230       240       250       260       270       280
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CH98 LC UA  AACACGGCCTCCCTGACAATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCTGCTCATCCC
CL900031    C........G.G.....T........A.....AAG...T.................T..T...ATT....

                   290       300       310       320       330
            ....|....|....|....|....|....|....|....|....|....|....|
CH98 LC UA  CTTTTCTTGGTAGTAATAATTGGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCT
CL900031    ...................A.....C................G..........T.
```

Supplemental Figure 4 - VL nucleotide sequence of monoclonal antibody CH98. The CH98 V_L/J_L sequence (CL900031) was aligned to its inferred unmutated ancestor sequence (CH98 LC UA). The CH98 LCDR1 (orange line) presents a 15-nucleotide deletion preceded by an activation-induced cytidine deaminase (AID) hotspot (AGT) (blue box). A TGT motif is also present near the start and the end of this deletion in the V_L unmutated gene sequence (red line). The N-nucleotides in-between the V_L and J_L sequences are shown with a blue line.

Supplemental Table 2 – CH98 and VRC01 binding profile to RSC3 protein, YU2 gp120 Env and Outer Domain protein 4.0 (OD4.0) and respective mutants.

	IC50 (µg/ml)							
	RSC3			YU2 gp120			OD4.0	
	WT	**G367R**	**Δ371I/P363N**	**WT**	**D368R**	**I420R**	**WT**	**I371N**
CH98	0.51	-	-	0.03	-	0.11	-	-
VRC01	0.02	0.46	-	0.01	0.71	0.04	0.53	-

Heavy Chain

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                 HFR1                HCDR1        HFR2         HCDR2                    HFR3
                (1-25)              (26-33)     (34-50)       (51-58)                 (59-96)

         _________________________ ________ _________________ ________ ______________________________________
         1       10        20         30         40        50          60        70        80        90
         .........|.........|..... ....|... ......|.........| ........ .|.........|.........|.........|......
CH103    QVQLQESGPGVVKSSETLSLTCTVS GGSMGGTY WSWLRLSPGKGLEWIGY IFHTGET. NYSPSLKGRVSISVDTSEDQFSLRLRSVTAADTAVYFC
CH98     EVQLVESGGGVVEPGKSLTVSCAAF GVNIDTYD FHWVRQAPGKGLEWVAC CSNSGEHS FNADIVKGRFSVSRDNMRNKMTLQMTRVMVDDTATYFC
          *** *** ***     *   *    *          * *  *******        **         * ** * * *      *    *   *** ***

                HCDR3               HFR4
              (97-118)           (119-129)

         ______________________ ___________
           100       110        120
         ...|.........|........ .|.........
CH103    ASLPRGQLVNAYFRN....... WGRGSLVSVTA
CH98     VKE.RGRHRTRDTLEFGRFFDS WGQGARVTVSS
             **       *    *    ** *  * *
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Light Chain

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                    LFR1             LCDR1        LFR2        LCDR2                LFR3
                   (1-26)           (27-35)      (36-50)     (51-57)              (58-89)

        __________________________ _________ _______________ _______ ________________________________
        1       10        20         30         40        50          60        70        80
        .........|.........|...... ...|..... ....|.........| ....... ..|.........|.........|.........
CH103   SYE....LTQPPSVSVSPGQTATITC SGA.STNVC WYQVKPGQSPEVVIF ENYKRPS GIPDRFSGSKSGSTATLTIRGTQAIDEADYYC
CH98    STGDQSALTQPAFVSESPGKSVTIAC LGATSDYVC WYQHYPGRAPKLLIF EANRRPS GVSDRFSGSKSGHTAALTISGLQSDDEADYYC
        *      ****  ** ***   ** *  ** *  ** ***  **  *   ** *   *** *  ********* ** *** * *  *******

            LCDR3       LFR4
          (90-101)    (102-111)

        ____________ __________
       90        100        110
        |.........|. ........|.
CH103   .QVWDSFS.TFV FGSGTQVTV.
CH98    CISPFLGSNKWV FGGGTRLTVL
               *   * ** **  **
```

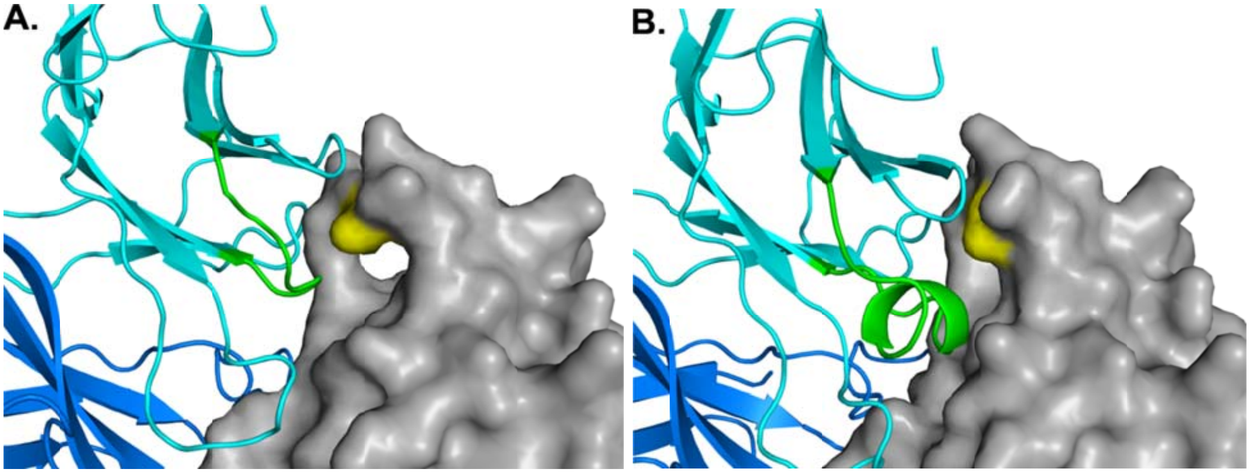
Supplemental Figure 5 - Amino acid alignment of CH98 and CH103 heavy and light chain V(D)J rearrangements. Conserved amino acids are indicated with asterisks.

Supplemental Table 3 - Percentage of homology between amino acid sequences of CH98 and CH103 heavy and light chain V(D)J rearrangements.

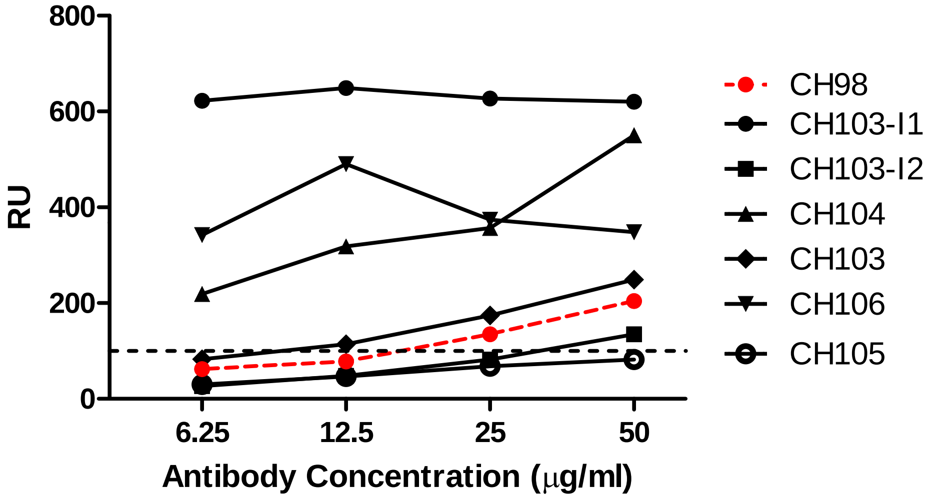
Heavy Chain			**Light Chain**		
HFR-1	11/25	44.0%	LFR-1	13/26	50.0%
HCDR-1	1/8	12.5%	LCDR-1	5/9	55.6%
HFR-2	9/17	52.9%	LFR-2	9/15	60.0%
HCDR-2	2/8	25.0%	LCDR-2	4/7	57.1%
HFR-3	14/38	36.8%	LFR-3	24/32	75.0%
HCDR-3	4/22	18.2%	LCDR-3	1/12	8.3%
HFR-4	5/11	45.5%	LFR-4	6/10	60.0%
Total	**46/129**	**35.7%**	**Total**	**62/111**	**55.9%**

Supplemental Table 4 - CH98 regions predicted to make contact with the observed YU2 gp120 core contact residues by threading CH98 onto the gp120-complexed CH103 crystal structure.

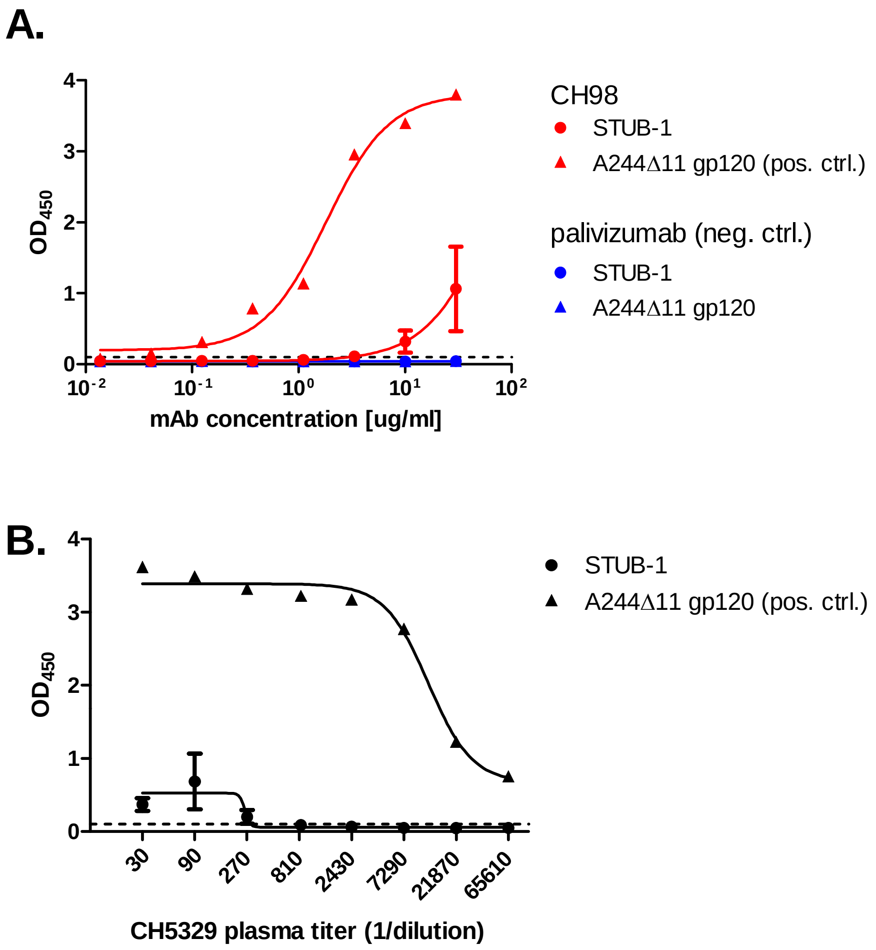
YU2 gp120 core contact residues	Predicted CH98 contact regions
K282	HCDR3, LCDR2
S365	HCDR1, HCDR3
S368	HCDR1, HCDR2, HCDR3, LCDR3
G473	HCDR1, HCDR3
D461	LCDR1, LCDR2



Supplemental Figure 6 - Predicted LCDR1 conformation of the mature and LCDR1 deletion-reverted CH98 bNAb. (A) Detail of the CH98 model (heavy chain in dark blue and light chain in light blue) based on threading onto the gp120-complexed CH103 crystal structure showing the short LCDR1 (green), which is predicted to make contact with residue 461 (yellow) of the gp120 molecule (gray). (B) Threading of the CH98 LCDR1 deletion-reverted antibody. Upon reversion of the 15-nucleotide deletion, the CH98 LCDR3 adopts the canonical conformation for a LCDR1 of length 14 as defined by North and colleagues (45) thus avoiding steric clashes but with a less favorable binding energy (see Results).



Supplemental Figure 7 - Binding to dsDNA of CH98 BnAb and antibodies from the CH103 CD4bs BnAb clonal lineage. CH98 BnAb binding to dsDNA was compared with the binding of mature CD4bs BnAb CH103, CH104, CH105, CH106 and maturational intermediate mAbs CH103-I1 and CH103-I2 described in Liao HX et al. Nature 496:469-476, 2013). Serial dilutions of each antibody (x-axis) were tested using the AtheNA Multi-Lyte ANA assay and binding to dsDNA is expressed as relative units (RU) (y-axis). CH98 is shown in red. The dotted line indicates the threshold for positivity. Results from an experiment representative of multiple experiments for each antibody are shown.



Supplemental Figure 8 - CH98 and CH5329 plasma binding to STUB-1. (A) CH98 BnAb (red) and negative control mAb palivizumab (blue) binding to STUB-1 (circles). HIV-1 A244Δ11 gp120 Env (triangles) was used as positive control for CH98 binding. The *x*-axis shows each monoclonal antibody concentration expressed as µg/ml and the *y*-axis shows the optical density reading at 450 nm. Experiments were run in duplicate, each point indicates the average and the error bars show the SEM. (B) CH5329 plasma binding to STUB-1 (circles) and HIV-1 A244Δ11 gp120 Env (triangles; positive control). The *x*-axis shows CH5329 plasma titer expressed as 1/plasma dilution (i.e. lowest dilution tested was 1:30) and the *y*-axis shows the optical density reading at 450 nm. Experiments were run in duplicate, each point indicates the average and the error bars show the SEM.

```
             1       10        20        30        40        50        60        70        80        90
             |--------|---------|---------|---------|---------|---------|---------|---------|---------|-------
Consensus    MGGKWSKRKMGGWPEVRERMRRTEPAAEGVGAASRDLEKYGALTSSNTVANNADCAWLAAQEEEEEEVGFPVTPQVPLRPMTYKGALDLSHFLKEKG
CH5329_3.1   -------------------------------------------------------------------------------------------------
CH5329_3.2   ----------------------------------------------------------------.-------R---------F---F----------
CH5329_3.4   ------------------------------------------------------------------------K-------------F----------
CH5329_3.5   -------------------------------------D----------------------------------K-------------F----------
CH5329_3.6   -------------------------------------D------------------------------------------------F----------
CH5329_3.7   -------------------------------------------------------------------------------------------------
CH5329_3.8   -------------------------------------------------------------------------------------------------
CH5329_3.10  -------------------------------------------------------------------------------------------------
CH5329_3.11  -------------------------------------------------------------------------------------------------
CH5329_3.12  -------------------------------------D-----------------------------------------------------------
CH5329_3.13  -------------------------------------------------------------------------------------------------
CH5329_3.14  ------------------------------------------------------------------------K-------------F----------
CH5329_3.15  ------------------------------------------------------------------------K-------------F----------
CH5329_3.16  -------------------------------------------------------------------------------------------------
CH5329_3.17  -------------------------------------D-----------------------------------------------------------
CH5329_3.19  -------------------------------------------------------------------------------------------------
CH5329_3.20  -------------------------------------------------------------------------------------------------
CH5329_3.21  --------------------------------------------------------------------------------------F----------
CH5329_3.23  -------------------------------------------------------------------------------------------------
CH5329_3.24  -------------------------------------------------------------------------------------------------
CH5329_3.26  -------------------------------------------------------------------------------------------------
CH5329_3.27  -------------------------------------------------------------------------------------------------
CH5329_3.28  -------------------------------------D----------------------------------K-------------F----------
CH5329_3.29  -------------------------------------------------------------------------------------------------
CH5329_3.30  -------------------------------------------------------------------------------------------------
CH5329_3.32  -------------------------------------------------------------------------------------------------
CH5329_3.34  -------------------------------------------------------------------------------------------------
CH5329_3.36  ----------------------------------------------------------------.-------R------------------------
CH5329_3.37  -------------------------------------D-----------------------------------------------------------
CH5329_3.38  -------------------------------------------------------------------------------------------------
CH5329_3.39  -------------------------------------------------------------------------------------------------
CH5329_3.40  -------------------------------------------------------------------------------------------------
CH5329_3.41  -------------------------------------K-----------------------------------------------------------
CH5329_3.42  -------------------------------------------------------------------------------------------------
CH5329_3.43  -------------------------------------------------------------------------------------------------
CH5329_3.44  -------------------------------------------------------------------------------------------------
CH5329_3.45  ------------------K------------------------------------------------------------------------------
CH5329_3.46  -------------------------------------------------------------------------------------------------
CH5329_3.47  --------------------------------------------------------------------------------------F----------
CH5329_3.48  -------------------------------------------------------------------------------------------------
CH5329_3.50  -------------------------------------------------------------------------------------------------
CH5329_3.51  -------------------------------------------------------------------------------------------------
CH5329_3.53  ----------D-------------------------------------------------------------K-------------F----------
CH5329_3.54  -------------------------------------------------------------------------------------------------
CH5329_3.55  ----------------------------------------------------------------.-------R-------------F----------
CH5329_3.56  -------------------------------------D--------H-------------------------K-------------F----------
CH5329_3.58  ----------------------------------------------------------------------------------------------G--
CH5329_3.59  -------------------------------------------------------------------------------------------------
CH5329_3.60  ------------------------------------------------------------------------R---------F---F----------
CH5329_3.61  ----------------------------------------------------------------.-------R---------F---F----------
CH5329_3.62  ----------------------------------------------------------------.-------R------------------------
CH5329_3.63  -------------------------------------------------------------------------------------------------
CH5329_3.71  -------------------------------------------------------------------------------------------------
CH5329_3.73  ----------------------------------------------------------------.-------R------------------------
CH5329_3.75  ----------------------------------------------------------------.-------R------------------------
CH5329_3.78  -------------------------------------------------------------------------------------------------
CH5329_3.80  -------------------------------------D----------------------------------K-------------F----------
CH5329_3.83  ----------------------------------------------------------------.-------R------------------------
CH5329_3.86  --------------------------------------------------------------------------------------F----------
CH5329_3.88  -------------------------------------------------------------------------------------------------
CH5329_3.95  -------------------------------------D-----------------------------------------------------------
CH5329_3.110 -------------------------------------------------------------------------------------------------
CH5329_3.112 ----------------------------------------------------------------.-------R------------------------
CH5329_3.142 --------------------------------------------------------------------------------------F----------
```

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              100       110       120       130       140       150       160       170       180       190
             --|---------|---------|---------|---------|---------|---------|---------|---------|---------|----
Consensus    GLQGLIYSQKRKDILDLWVYHTQGYFPDWQNYTPGPGTRFPLTFGWCFKLVPVEPEEVEQANEGENNVLLHPMSQHGMDDPEKEVLKWVFDSKLAFH
CH5329_3.1   -------------------------------------------------------------------------------------------------
CH5329_3.2   --D----------------------------------------------------------------------------------------------
CH5329_3.4   --D--------------------------------------------------------K-----------------------------------LR
CH5329_3.5   --D---------------------F----------------------------------K-----------------------------------LR
CH5329_3.6   --H----------------------------------------------------------------------------------------------
CH5329_3.7   -------------------------------------------------------------------------------------------------
CH5329_3.8   --E----------------------------------------------------------------------------------------------
CH5329_3.10  -------------------------------------------------------------------------------------------------
CH5329_3.11  -------------------------------------------------------------------------------------------------
CH5329_3.12  -------------------------------------------------------------------------------------------------
CH5329_3.13  -------------------------------------------------------------------------------------------------
CH5329_3.14  --D----------------------------------------------------------------------------------------------
CH5329_3.15  --D---------------------F------------------------------------------------------------------------
CH5329_3.16  -------------------------------------------------------------------------------------------------
CH5329_3.17  -------------------------------------------------------------------------------------------------
CH5329_3.19  -------------------------------------------------------------------------------------------------
CH5329_3.20  ---------------------------------------------------------------------V---------------------------
CH5329_3.21  --D-----K---------------F------------------------------------------------------------------------
CH5329_3.23  -------------------------------------------------------------------------------------------------
CH5329_3.24  --E----------------------------------------------------------------------------------------------
CH5329_3.26  -------------------------------------------------------------------------------------------------
CH5329_3.27  -------------------------------------------------------------------------------------------------
CH5329_3.28  --D---------------------F----------------------------------K-----------------------------------LR
CH5329_3.29  -------------------------------------------------------------------------------------------------
CH5329_3.30  --E----------------------------------------------------------------------------------------------
CH5329_3.32  -------------------------------------------------------------------------------------------------
CH5329_3.34  -------------------------------------------------------------------------------------------------
CH5329_3.36  --E---------------------------------------------------------T------------------------------------
CH5329_3.37  -------------------------------------------------------------------------------------------------
CH5329_3.38  -------------------------------------------------------------------------------------------------
CH5329_3.39  -------------------------------------------------------------------------------------------------
CH5329_3.40  -------------------------------------------------------------------------------------------------
CH5329_3.41  -------------------------------------------------------------------------------------------------
CH5329_3.42  ------------------------------D------------------------------------------------------------------
CH5329_3.43  --E----------------------------------------------------------------------------------------------
CH5329_3.44  -------------------------------------------------------------------------------------------------
CH5329_3.45  -------------------------------------------------------------------------------------------------
CH5329_3.46  -------------------------------------------------------------------------------------------------
CH5329_3.47  --D--------------------------------------------------------K-------------------------------------
CH5329_3.48  -------------------------------------------------------------------------------------------------
CH5329_3.50  --E----------------------------------------------------------------------------------------------
CH5329_3.51  -------------------------------------------------------------------------------------------------
CH5329_3.53  --D--------------------------------------------------------K-------------------------------------
CH5329_3.54  -------------------------------------------------------------------------------------------------
CH5329_3.55  --D----------------------------------------------------------------------------------------------
CH5329_3.56  --D---------------------F----------------------------------K-----------------------------------LR
CH5329_3.58  --E----------------------------------------------------------------------------------------------
CH5329_3.59  -------------------------------------------------------------------------------------------------
CH5329_3.60  --D----------------------------------------------------------------------------------------------
CH5329_3.61  --D--------------------------------------------------------K-------------------------------------
CH5329_3.62  --E----------------------------------------------------------------------------------------------
CH5329_3.63  -------------------------------------------------------------------------------------------------
CH5329_3.71  --E----------------------------------------------------------------------------------------------
CH5329_3.73  --E----------------------------------------------------------------------------------------------
CH5329_3.75  --E----------------------------------------------------------------------------------------------
CH5329_3.78  -------------------------------------------------------------------------------------------------
CH5329_3.80  --D---------------------F----------------------------------K-----------------------------------LR
CH5329_3.83  --E----------------------------------------------------------------------------------------------
CH5329_3.86  --H----------------------------------------------------------------------------------------------
CH5329_3.88  -------------------------------------------------------------------------------------------------
CH5329_3.95  -------------------------------------------------------------------------------------------------
CH5329_3.110 -------------------------------------------------------------------------------------------------
CH5329_3.112 --E----------------------------------------------------------------------------------------------
CH5329_3.142 --H----------------------------------------------------------------------------------------------
```

```
                 200
             -----|---------
Consensus    HVAQELHPEYYSKDR
CH5329_3.1   ---------------
CH5329_3.2   ---------------
CH5329_3.4   -----M---------
CH5329_3.5   -----M---------
CH5329_3.6   ---------------
CH5329_3.7   ---------------
CH5329_3.8   ---------------
CH5329_3.10  ---------------
CH5329_3.11  ---------------
CH5329_3.12  ---------------
CH5329_3.13  ---------------
CH5329_3.14  ---------------
CH5329_3.15  ---------------
CH5329_3.16  ---------------
CH5329_3.17  ---------------
CH5329_3.19  ---------------
CH5329_3.20  ---------------
CH5329_3.21  ---------------
CH5329_3.23  ---------------
CH5329_3.24  ---------------
CH5329_3.26  ---------------
CH5329_3.27  ---------------
CH5329_3.28  -----M---------
CH5329_3.29  ---------------
CH5329_3.30  ---------------
CH5329_3.32  ---------------
CH5329_3.34  ---------------
CH5329_3.36  ---------------
CH5329_3.37  ---------------
CH5329_3.38  ---------------
CH5329_3.39  ---------------
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CH5329_3.41  ---------------
CH5329_3.42  ---------------
CH5329_3.43  ---------------
CH5329_3.44  ---------------
CH5329_3.45  ---------------
CH5329_3.46  ---------------
CH5329_3.47  ---------------
CH5329_3.48  ---------------
CH5329_3.50  ---------------
CH5329_3.51  ---------------
CH5329_3.53  ---------------
CH5329_3.54  ---------------
CH5329_3.55  ---------------
CH5329_3.56  -----M---------
CH5329_3.58  ---------------
CH5329_3.59  ---------------
CH5329_3.60  ---------------
CH5329_3.61  ---------------
CH5329_3.62  ---------------
CH5329_3.63  ---------------
CH5329_3.71  ---------------
CH5329_3.73  ---------------
CH5329_3.75  ---------------
CH5329_3.78  ---------------
CH5329_3.80  -----M---------
CH5329_3.83  ---------------
CH5329_3.86  ---------------
CH5329_3.88  ---------------
CH5329_3.95  ---------------
CH5329_3.110 ---------------
CH5329_3.112 ---------------
CH5329_3.142 ---------------
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Supplemental Figure 9 - Nef gene sequences from subject CH5329. Analysis of 64 3'-half genome amino acid sequences generated by the single genome amplification method showed an intact *nef* gene in all sequences.

Supplemental Table 5 - Viruses used in neutralization assays.

Isolate	Accession No.	Clade	Origin	Contributor(s)
Q23.17	AF004885	A	Kenya	Julie Overbaugh
Q842.d12	AF407160	A	Kenya	Julie Overbaugh
Q168.a2	AF407148	A	Kenya	Julie Overbaugh
92RW020.2	EU855131	A	Rwanda	
MN.3	HM215430	B	USA	David C. Montefiori
SF162.LS	EU123924	B	USA	Leonidas Stamatatos
MW965.26	U08455	C	Malawi	Beatrice Hahn, Feng Gao
Bal.26	DQ318211	B	USA	John Mascola
Bx08.16	GQ855765	B	France	David C. Montefiori
SS1196.1	AY835442	B	USA	David C. Montefiori
6535.3	AY835438	B	USA	David C. Montefiori
6240_08_TA5_4622	EU289190	B	USA	George Shaw, Truman Grayson
6244_13_B5_4576	EU289191	B	USA	George Shaw, Truman Grayson
AC10.0.29	AY835446	B	USA	David C. Montefiori
CAAN5342.A2	AY835452	B	USA	Beatrice Hahn, Denise Kothe
PVO.4	AY835444	B	Italy	David C. Montefiori
QH0692.42	AY835439	B	Trinidad	David C. Montefiori
REJO4541.67	AY835449	B	USA	Beatrice Hahn, Jesus Salazar
RHPA4259.7	AY835447	B	USA	Beatrice Hahn, Jesus Salazar
SC422661.8	AY835441	B	Trinidad	Beatrice Hahn
THRO4156.18	AY835448	B	USA	Beatrice Hahn, Denise Kothe
TRJO4551.58	AY835450	B	USA	Beatrice Hahn, Xiping Wei
TRO.11	AY835445	B	Italy	David C. Montefiori
WEAU_d15_410_5017	EU289202	B	USA	George Shaw, Truman Grayson
WITO4160.33	AY835451	B	USA	Beatrice Hahn, Jesus Salazar
6101.10	AY835434	B	USA	David C. Montefiori
1394C9G1(Rev-)	FJ444529	C	Malawi	Beatrice Hahn, Jesus Salazar
246F C1	FJ496194	C	Zambia	Beatrice Hahn, Truman Grayson
BF1266.431a	HM215360	C	Malawi	Ronald Swanstrom, Elizabeth Russell
CAP45.2.00.G3	DQ435682	C	S. Africa	Lynn Morris
Ce0682_E4	FJ444325	C	Malawi	Ronald Swanstrom, Li-Hua Ping, Jeffrey Anderson
Ce2060_G9	FJ444600	C	Malawi	Ronald Swanstrom, Li-Hua Ping, Jeffrey Anderson
Ce703010054_2A2	FJ443808	C	Malawi	Ronald Swanstrom, Li-Hua Ping, Jeffrey Anderson
Du156.12	DQ411852	C	S. Africa	David C. Montefiori
TV1.21	HM215437	C	S. Africa	David C. Montefiori
Du422.1	DQ411854	C	S. Africa	David C. Montefiori
Du151.2	DQ411851	C	S. Africa	David C. Montefiori
3016.v5.c45	HM215283	D	Tanzania	Jerome Kim, Sodsai Tovanabutra, Eric Sanders-Buell
P0402_c2_11	EU885759	G	Portugal	Michael Thomson, Ana Revilla, Elena Delgado, Yolanda Vega
CM244.ec1	AY713425	CRF01_AE	Thailand	Jerome Kim, Sodsai Tovanabutra, Eric Sanders-Buell
TH023.6	AF009393	CRF01_AE	Thailand	Jerome Kim, Sodsai Tovanabutra, Eric Sanders-Buell
NP03.13	KC822430	CRF01_AE	Thailand	Jerome Kim/ Sodsai Tovanabutra/ Eric Sanders-Buell